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Viral Infections of *Macrobrachium* spp.: Global Status of Outbreaks, Diagnosis, Surveillance, and Research

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Key words: *Macrobrachium rosenbergii*, white tail disease, *Macrobrachium rosenbergii* nodavirus (*Mr*NV), extra small virus-like particle (XSV), host range, vertical transmission

Abstract

Macrobrachium rosenbergii, a global and economically important cultured freshwater prawn, is farmed on a large scale in many countries. Compared to penaeid shrimps, *M. rosenbergii* is a moderately disease-resistant species. However, viruses 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 (*Mr*NV), and extra small virus-like particle (XSV) have been reported and are responsible for economic losses to freshwater prawn culture. *Mr*NV and XSV, the causes of white tail disease (WTD), have been reported as dangerous viruses to *M. rosenbergii*, resulting in 100% mortality in postlarvae and juveniles within five days of infection. Clinical signs of WTD include lethargy and opaqueness of the abdominal muscle. Various aspects of WTD are discussed in this paper, including tissue tropism of the causative viruses, host range, virus structure, vertical transmission, pathogenicity, and the possibility of multiplying *Mr*NV and XSV in a mosquito cell line.

Introduction

The giant freshwater prawn *Macrobrachium rosenbergii*, commonly known as 'scampi', is an economically important cultured freshwater prawn throughout the world. It is farmed on a large scale in many countries. *Macrobrachium rosenbergii* is native to Thailand and other Southeast Asian countries including Vietnam, Kampuchea, Malaysia, Myanmar, Bangladesh, India, Sri Lanka, and the Philippines. It is produced in Israel, Japan, Taiwan, and some African, Latin American, and Caribbean countries (New, 1990). 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

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potential for freshwater prawn culture. Scampi can be cultivated for export through monoculture in existing or new ponds or together with compatible freshwater fishes in existing ponds. Freshwater prawn farming in India has increased dramatically in the last decade with total production reaching an all-time high of 20,000 tons in 2002, third rank in world prawn production. 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.

All of today's farmed freshwater prawns belong to the genus *Macrobrachium*. The three main species cultured commercially until 2000 were *M. rosenbergii* in many countries, the oriental river prawn *M. nipponense* (De Haan, 1849) in China (Wang and Qianhong, 1999; Kutty et al., 2000), and the monsoon river prawn *M. malcolmsonii* (Edwards, 1844) in India (Kanaujia et al. 1997; Kutty et al., 2000). Like other animals, prawns are affected by viruses, bacteria, fungi, and metazoan parasites. Generally, *M. rosenbergii* is considered moderately disease-resistant in comparison to penaeid shrimp. Among the pathogens, prawn viruses are very important and responsible for huge economic losses, particularly in the hatchery and nursery phases. Reported prawn viruses include *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 (*Mr*NV), and extra small virus-like particle (XSV).

Macrobrachium Hepatopancreatic Parvo-Like Virus (MHPV)

The first reported virus in *M. rosenbergii* was MHPV. It appeared in postlarvae in a single hatchery in the Malaysian peninsula (Anderson et al., 1990). No viral diseases had been reported in *M. rosenbergii* before this work. The virus resembles hepatopancreatic parvovirus (HPV) in penaeid shrimp and similarities include the presence of basophilic inclusions in hepatopancreatic tubular epithelial cells, particle size (29 nm against 22-24 nm), DNA content, and intranuclear replication. This viral infection was not associated with mortality and laboratory stress conditions did not increase its intensity or prevalence. No further work was carried out on MHPV after its report.

Macrobrachium Muscle Virus (MMV)

An epizootic disease similar to idiopathic muscle necrosis (IMN) was reported in postlarvae in Taiwan (Tung et al., (1999). Infected postlarvae showed white opaque areas in abdominal segments accompanied by weakening of feeding and swimming activity. Histologically, there were cytoplasmic inclusion bodies in the necrotic muscle of infected postlarvae. Electron microscopy revealed icosahedral virus particles in the cytoplasm of necrotic cells as well as aggregations of viral particles in the inclusion body. This virus is temporarily named *Macrobrachium* muscle virus (MMV). It differs from similar viruses by being found only in *M. rosenbergii* and in striated muscle, and by forming basophilic inclusions within the cytoplasm. No further work was carried out on MMV after its first report.

Infectious Hypodermal and Haematopoietic Necrosis Virus (IHHNV)

High mortalities (up to 100%) were encountered in hatchery-reared postlarvae of *M. rosenbergii* in southern Taiwan (Hsieh et al., 2006). Histopathological investigation revealed the presence of eosinophilic intranuclear inclusion bodies in the hepatopancreatic tubular epithelial cells of infected postlarvae. Infected postlarvae were screened by PCR using primers specific to IHHNV, HPV, WSSV, taura syndrome virus (TSV), yellow head virus (YHV), *Mr*NV, and XSV; the samples reacted only to IHHNV primers for amplifying a product of 389 bp specific to IHHNV. The infection was further confirmed by *in situ* hybridization using a DIG-labeled probe specific to IHHNV. No further work was carried out on IHHNV after its first report.

White Spot Syndrome Virus (WSSV)

WSSV is highly pathogenic and responsible for huge economic losses in the shrimp culture industry throughout the world (Chang et al., 1998). Almost all species of penaeid shrimp are susceptible to WSSV. The major species naturally infected by this virus include *Penaeus monodon, P. indicus, P. japonicus, P. chinensis, P. merguensis, P. aztecus, P. stylirostris, P. vannamei, P. duorarum*, and *P. setiferus* (Lightner, 1996).

WSSV is highly pathogenic not only to penaeid shrimp, but also to marine crabs and freshwater prawns and crabs (Cai et al., 1995; Lo et al., 1996; Wang et al., 1998; Chen et al., 2000; Sahul Hameed et al., 2000, 2001, 2002). *Macrobrachium rosenbergii* is not a natural host of WSSV, but the syndrome was experimentally induced in this species via different routes of infection. *Macrobrachium rosenbergii* is tolerant to WSSV. The virus failed to produce mortality although some organs of infected animals were WSSV-positive when tested by PCR and Western blot analysis (Rajendran et al., 1999; Sahul Hameed et al., 2000; Kiran et al., 2002; Yoganandhan and Sahul Hameed, 2007). WSSV changed pathogenicity after passaging through *M. rosenbergii*. Whereas it caused 95% mortality in experimental *P. monodon* before passaging through *M. rosenbergii*, it caused only 5% mortality after passaging (Waikhom et al., 2006). The reason for this change is unknown but might be due to the inactivation of WSSV passaged through *M. rosenbergii* (Waikhom et al., 2006).

Transcriptional and translational analyses of WSSV from organs of WSSV-injected *M. rosenbergii* by reverse transcriptase polymerase chain reaction (RT-PCR) and Western blot assays revealed transient expression of the VP28 gene up to 4 days post infection (dpi) but from 5 dpi onwards the gene was undetectable (Yoganandhan and Sahul Hameed, 2007). Inoculum prepared from WSSV-injected prawns 5 dpi caused 100% mortality in *P. monodon* while inoculum prepared later than 5 dpi failed to cause mortality in *P. monodon* (Sarathi et al., 2008). These results coincide with RT-PCR and Western blot results and with Waikhom et al. (2006) who observed very low mortality in *P. monodon* injected with WSSV passaged through *M. rosenbergii*. We observed the clearance of WSSV in WSSV-injected *M. rosenbergii* by PCR, Western blot, and bioassay, indicating that some anti-viral mechanism or clearing mechanism against WSSV may be involved (Sarathi et al., 2008).

Macrobrachium rosenbergii Nodavirus (MrNV) and Extra Small Virus-like Particle (XSV)

A new disease named white tail disease (WTD) was reported from the island of Guadeloupe in 1995 (Arcier et al., 1999). It was next reported in Martinique, French West Indies (1999), Taiwan (1999), the People's Republic of China (2003), India (2003) and, most recently, Thailand.

Clinical signs and histopathology. The clinical signs of WTD include lethargy and opaqueness of the abdominal muscle that gradually extends from the center to the anterior and the posterior sections of the muscle (Fig. 1). Degeneration of the telson and uropods is observed in severe cases. There are basophilic cytoplasmic inclusions with a diameter of 1-40 mm in striated muscles of the abdomen, cephalothorax, and intratubular connective tissue of the hepatopancreas. No viral inclusions were observed in epithelial cells of the hepatopancreatic tubules or in midgut mucosal epithelial cells (Arcier et al., 1999).

Causative organisms and pathogenicity. Two viruses, *Macrobrachium rosenbergii* nodavirus (*Mr*NV) and Extra Small Virus-like Particle (XSV), are responsible for WTD (Bonami et al., 2005). *Mr*NV is a small, icosahedral, non-enveloped particle, 26-27 nm in diameter. It contains two single-stranded RNAs: RNA1 (2.9 kb) and RNA2 (1.26 kb). Its capsid contains a single polypeptide of 43 kDa. With these characteristics, it is closely related to the *Nodaviridae* family. XSV is also a non-enveloped icosahedral virus, 15 nm in diameter, with a linear ssRNA genome of 0.9 kb encoding two overlapping structural proteins of 16 and 17 kDa (Bonami et al., 2005). 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 *Mr*NV a helper virus.

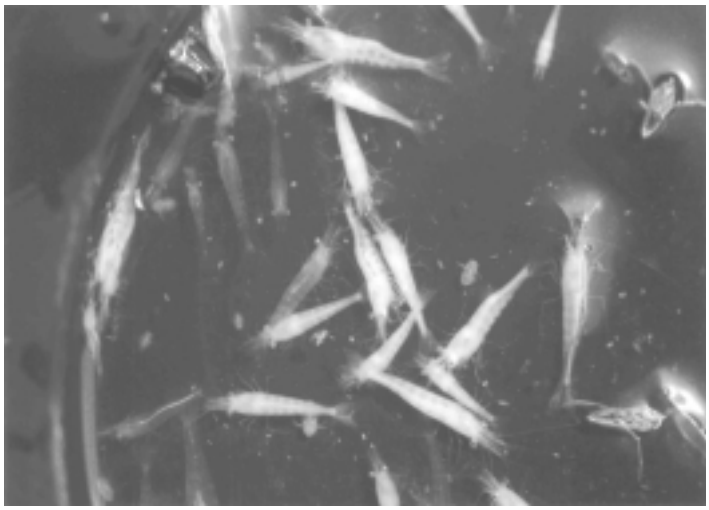

Fig. 1. Opaqueness of the abdominal muscle in hatchery-reared *Macrobrachium rosenbergii* postlarvae infected with white tail disease (WTD).

These two viruses caused 100% mortality in postlarvae 12 days post infection by immersion. In the virus-infected group, postlarvae started showing whitish muscles 7 dpi and reached the highest proportion at 12 dpi. When infected by the intramuscular route, the viruses failed to cause mortality in adult prawns. RT-PCR analysis confirmed the infection in experimentally infected postlarvae (Qian et al., 2003; Sahul Hameed et al., 2004) and in gill tissue, head muscle, stomach, intestine, heart, hemolymph, pleopods, ovaries, and tail muscles of experimentally injected adult prawns (Sahul Hameed et al., 2004).

*Mr*NV and XSV were purified and postlarvae were challenged with different combinations of these two viruses by immersion (Zhang et al., 2006). Clinical signs of WTD were observed in postlarvae challenged with combinations containing a relatively high proportion of *Mr*NV and low proportion of XSV. In contrast, there was little sign of WTD in postlarvae challenged with a higher proportion of XSV than *Mr*NV, indicating that *Mr*NV plays a key role in WTD of *M. rosenbergii*.

*Diagnostic methods. Mr*NV and XSV can be detected by genome-based and protein-based methods. Genome-based methods include dot-blot hybridization, *in situ* hybridization, and RT-PCR (Sri Widada et al., 2003; Sahul Hameed et al., 2004). Dot-blot hybridization was applied to archived samples and could detect *Mr*NV at a level of 0.8 pg of viral RNA (Sri Widada et al., 2003). *In situ* hybridization using an *Mr*NV probe was used to study the tropism of *Mr*NV in infected animals (Sri Widada et al., 2003). Results revealed that *Mr*NV infection was confined to striated muscle tissue of the abdomen, cephalothorax, and appendages. RT-PCR is the most sensitive diagnostic method for detecting *Mr*NV and XSV (Fig. 2). Primers have been designed by different workers to detect *Mr*NV and XSV in infected samples (Sri Widada et al., 2003; Sahul Hameed et al., 2004).

To avoid having to carry out two RT-PCR reactions, one for each virus, we developed a modified method, one-step multiplex RT-PCR (mRT-PCR), to simultaneously detect both in a single tube (Yoganandhan et al., 2005). These concurred with bands obtained in separate RT-PCR assays, one for each virus. In addition, loop-mediated isothermal amplification (LAMP) was used to detect *Mr*NV and XSV (Pillai et al., 2006).

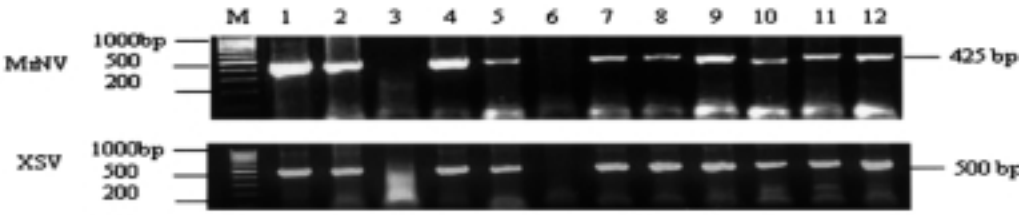

Fig. 2. RT-PCR detection of *Macrobrachium rosenbergii* nodavirus (*Mr*NV) and extra small virus-like particle (XSV) in organs of experimentally infected adult giant freshwater prawns (*Macrobrachium rosenbergii*). M = marker, 1 = hemolymph, 2 = gill tissue, 3 = hepatopancreas, 4 = heart, 5 = stomach, 6 = eyestalk, 7 = head muscle, 8 = abdominal muscle, 9 = tail muscle, 10 = ovarian tissue, 11 = intestine, 12 = pleopods.

A protein-based sandwich enzyme-linked immunosorbent assay (S-ELISA) was developed to detect *Mr*NV using polyclonal antiserum raised against purified *Mr*NV (Romestand and Bonami, 2003). Monoclonal antibodies against *Mr*NV and a triple antibody S-ELISA was applied using these mAbs (Qian et al., 2006).

Replication of *Mr*NV and XSV was investigated in apparently healthy C6/36 subclones of the *Aedes albopictus* cell line. Results revealed that C6/36 cells are susceptible to these viruses. Replication of the viruses in the C6/36 cell line was confirmed using RT-PCR. The inoculum used to infect the cells was positive only by nested RT-PCR and yielded one-step RT-PCR positive at 72 hours post infection. We confirmed the successful propagation of *Mr*NV and XSV in C6/36 cells by showing that the supernatant medium from fifth passage cultures is able to initiate *Mr*NV/XSV infection in *M. rosenbergii* postlarvae. This study opens the way for future studies on viral binding and interaction of *Mr*NV and XSV with host cells (Sudhakaran et al., 2007b).

Vertical and horizontal transmission. Experiments were carried out to determine the possibility of vertical transmission of *Mr*NV and XSV in *M. rosenbergii* (Sudhakaran et al., 2007a). Prawn broodstock inoculated with *Mr*NV and XSV by oral or immersion challenge survived without any clinical signs of WTD. Brooders spawned 5-7 dpi and eggs hatched. The survival rate of larvae gradually decreased, and 100% mortality was observed at the postlarvae stage. Whitish muscle, the typical sign of WTD, was seen in advanced larvae stages. Ovarian tissue and fertilized eggs were positive for *Mr*NV/XSV when tested by RT-PCR whereas larvae were positive by nested RT-PCR (Sudhakaran et al., 2007a).

In horizontal transmission experiments, five developmental stages of *Artemia* were exposed to *Mr*NV and XSV by immersion and oral routes to determine whether *Artemia* acts as a reservoir or carrier of the viruses (Sudhakaran et al., 2006b). 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* postlarvae fed *Artemia* nauplii exposed to *Mr*NV and XSV by either immersion or oral challenge routes while no mortality was observed in postlarvae fed virus-free *Artemia*. RT-PCR analysis of the *M. rosenbergii* postlarvae confirmed the presence of *Mr*NV 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* postlarvae.

Host susceptibility. The susceptibility of three species of marine shrimp (*Penaeus indicus, P. japonicus*, and *P. monodon*) to *Mr*NV and XSV was tested by oral route and intramuscular injection (Sudhakaran et al., 2006a). The viruses failed to produce mortality in the shrimp but RT-PCR analysis revealed the presence of *Mr*NV and XSV in the gills, abdominal muscle, stomach, intestine, and hemolymph of shrimp injected with the viruses. Re-inoculation using inoculum of *Mr*NV

and XSV prepared from the marine shrimp caused 100% mortality in freshwater prawn postlarvae, with moribund postlarvae testing positive for these viruses by RT-PCR. Our study indicates the possibility that marine shrimp act as a reservoir for *Mr*NV and XSV and that these viruses maintain virulence in the shrimp tissue system.

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 the present 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 center 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 *Mr*NV and XSV (unpublished data).

Control measures. RT-PCR is available for commercial use. Therefore, broodstock and seed screening are strongly encouraged to prevent WTD. Broodstock or seed that test positive for WTD should be discarded by proper zoo-sanitary methods. Usual sanitation and control protocols for viral infections are recommended.

Future Work

Future work should concentrate on the relationship between the two viruses to determine which carries the pathogenicity and the precise role of each virus in the disease. Are the two viruses always associated and is XSVdependent on *Mr*NV or can it use another helper virus? Further work is needed on determining the host range of WTD.

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