Fishery Technology
2009, Vol. 46(1) pp : 45 - 52

White Tail Disease: A Viral Disease of Giant Freshwater Prawn, *Macrobrachium rosenbergii* (deMan)

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Virus borne diseases are extremely difficult to control and constitute one of the major problems of prawn farming. Till recently, the giant freshwater prawn, *Macrobrachium rosenbergii* was regarded as disease resistant. But there have been recent reports on white tail disease (WTD), a virus borne disease of *M.rosenbergii* affecting the aquaculture industry of India. In this review, clinical signs, causative agents, mode of transmission and various diagnostic methods of white tail disease are discussed.

Key words: ***Macrobrachium rosenbergii*, White tail disease (WTD), Molecular diagnostic methods**

As aquaculture expands and intensifies, new diseases emerge. The giant freshwater prawn *Macrobrachium rosenbergii* (deMan), commonly known as scampi, is one of the most economically important farmed palaemonids cultured in many parts of the world including India. The annual production of cultured freshwater prawns increased from 7140 MT in the year 2000 to 30,450 MT in 2003 in India and the country ranks third in total production after China and Vietnam (Bojan *et al.*, 2003; Nambudiri, 2003). Now this species is under serious threat due to a virus borne disease termed white tail disease (WTD). WTD was first reported from the French West Indies (Arcier *et al.*, 1999). It was then reported in Taiwan (Tung *et al.*, 1999) and China (Qian *et al.*, 2003). It has been observed in hatcheries and nursery ponds in different parts of India and has caused high mortalities and economic losses. (Sahul Hameed *et al.*, 2004 a). The causative agent of WTD was identified as *Macrobrachium rosenbergii* nodavirus (*Mr*NV) (Arcier *et al.*, 1999). Qian *et al.* (2003) have subsequently reported the occurrence of an additional virus like particle, extra small virus (XSV) associated with *Mr*NV. Sahul Hameed *et al.* (2004a) have reported the occurrence of these two viruses in larvae and post larvae (PL) of *M. rosenbergii* infected with WTD in India.

White tail disease and its clinical signs

The clinical signs of this disease include lethargy and opaqueness of abdominal muscle in infected larvae, post larvae and adults (Qian *et al.*, 2003). This opaqueness gradually extends to both anterior and posterior sides and leads to the degeneration of telson and uropods in extreme cases. Whitish appearance of the tail is the prominent clinical sign and therefore the disease is named white tail disease (WTD) (Fig.1). The mortality may reach 100% within two or three days after the appearance of the clinical sign of opaqueness. A few post larvae presenting these signs survive and survivors seem to grow normally in grow out ponds.

Causative agents

The two viruses were separated, purified and subsequently characterized. *Mr*NV is a small icosahedral non-enveloped particle, 26-27 nm in diameter, identified in the cytoplasm of connective cells. It contains a genome consisting of two single stranded

Fig. 1 Hatchery reared post larvae of *Macrobrachium rosenbergii* infected with white tail disease (Note the opaqueness of abdominal muscle).

positive sense RNA segments, RNA1 of 2.9 kb, which encodes the viral part of the RNA-dependent RNA polymerase (RdRp) and RNA2 of 1.3 kb that encodes the capsid protein. The capsid contains a single polypeptide of 43 kDa (cp-43) (Bonami *et al.*, 2005). In infected cells' RNA3, a sub genomic transcript of RNA1, is also present. These characteristics and the partial sequence of cloned fragment of RNA1 suggest that this agent is a member of the family Nodaviridae but with differences from both the genera *Alphanodavirus* and *Betanodavirus* (Bonami *et al.*, 2005). XSV is also an icosahedral virus with a diameter of 14-16 nm. Its genome consists of a linear single stranded positive sense RNA about 0.9 kb and a capsid protein, cp-17 (Sri Widada *et al.*, 2004). Surprisingly, it does not have any gene that codes for viral RNA replication. Thus, XSV may be a satellite virus dependent on *Mr*NV for its replication (Sri Widada *et al* ., 2004). *Mr*NV plays a key role in WTD of *M. rosenbergii*. The linear correlation between *Mr*NV and XSV genome copies in infected prawns confirmed that XSV is a satellite virus, dependent on *Mr*NV, but its role in pathogenicity of WTD remains unclear (Zhang *et al.*, 2006).

Analysis of the nucleotide sequence of *Mr*NV isolated in India showed 98% nucleotide and 99% amino acid sequence identity with the isolate from West Indies (Shekar *et al.*, 2006). The authors suggested that the two isolates are probably very closely related, isolated, if not the same. A similar result was obtained by Tripathy *et al.* (2006) with *Mr*NV capsid protein gene sequence. The nucleotide sequence of the partial genome of *Mr*NV RNA-2 of Taiwan isolates revealed a 97% identity with an Indian isolate (Wang *et al.*, 2008). The Taiwan isolate of XSV revealed 98% and 97% identity with that from China and India, respectively (Wang *et al.*, 2007). Phylogenetic analysis by them indicated that XSV-Taiwan isolate was more closely related to the Chinese rather than the Indian isolates. In a similar study, Sudhakaran *et al.* (2008) found the Indian isolate of XSV to be more closely related to Taiwanese and Chinese isolates than French and Thai isolates, leading them to suggest that the possible origin of white tail disease in India was from Taiwan and China through import of prawn seed.

Mode of transmission and life cycle

The successful artificial infection and rapid spread of epizootics during post larval period confirmed the possibility of 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 brood stock inoculated with *MrNV* and XSV by oral or immersion challenge survived. However, the survival rate of larvae from the brood stock gradually decreased, and cent percent mortality was observed at the post larval stage. Whitish muscles, the typical sign of WTD, were seen in advanced larval developmental stages. The ovarian tissue and fertilized eggs were found to be positive for *Mr*NV and XSV by RT-PCR (Sahul Hameed *et al.*, 2004b). These results show that the two viruses are transmitted vertically too, from mother directly to larvae in *M. rosenbergii*. Studies showed that Artemia

could be a possible vector for horizontal transmission of *Mr*NV and XSV to post larvae (Sudhakaran *et al.*, 2006a). A study that investigated the susceptibility of three species of marine shrimp *Fenneropenaeus indicus, Marsupenaeus japonicus* and *Penaeus monodon* to WTD showed that these shrimps were not susceptible to the two viruses. However, reinoculation studies with inoculum prepared using these inoculated marine shrimp caused 100% mortality in post larvae of *M. rosenbergii,* suggesting that marine shrimp may also serve as a reservoir for these viruses (Sudhakaran *et al.*, 2006b). Replication of these viruses occur in the cytoplasm of the cell. *Mr*NV and XSV can be successfully propagated in C6/36 mosquito cell line and electron microscopic observation of ultra thin sections revealed viral replication in the cytoplasm (Sudhakaran *et al.*, 2007b). The associated factors of WTD (temperature, salinity etc) are not yet known.

Disease diagnostic methods

As there are no effective treatments to viral pathogens, the spread and impact of white tail disease can be minimized only through adoption of better management practices in hatcheries and farms. Early diagnosis of pathogens in hatcheries is the only way to prevent the development of disease. So it is essential that sensitive, specific and rapid diagnostic method be developed for early detection of both pathogenic agents of the disease (Pillai *et al.*, 2006). Various diagnostic methods have been developed and standardized for detection of *Mr*NV and XSV in *M. rosenbergii.*

1. Traditional methods
2. Immuno diagnostic methods
3. Genome based detection methods

1. Traditional methods

Traditional methods for identifying pathogens are mainly based on phenotypic characters.

Clinical signs

As mentioned earlier, *Mr*NV infected animals are mainly diagnosed from the whitish coloration of their tail. However, this method is non-specific and there are chances of misinterpretation as many other diseases can cause the same signs. This method, therefore, cannot be used for systematic viral detection.

Histopathology

Histopathological changes in affected post larvae (PL) were characterized predominantly by pale to darkly basophilic, often reticulated cytoplasmic inclusions in the connective tissue cells of most organs and tissues (Arcier *et al.*, 1999). Detection of viral particles by transmission electron microscopy is too cumbersome to be used for routine screening. Moreover, it requires a high level of technical skill.

2. Immuno diagnostic methods

Sandwich enzyme linked immunosorbent assay (S-ELISA)

A sandwich enzyme linked immunosorbent assay was developed to improve diagnosis of white tail disease of *M. rosenbergii* (Romestand & Bonami, 2003). Polyclonal antibodies were produced by immunization of Balb/c mice using a purified suspension of the virus and IgG anti-*Mr*NV were purified from the ascitic fluid. A sandwich method was successfully developed, coating first with unlabelled antibody and detecting trapped antigens with a second biotinylated antibody. Reaction was demonstrated using an avidin-peroxidase conjugate. Tissue extracts from *M. rosenbergii* infected with *Mr*NV were successfully identified in an individual ELISA, thus confirming the validity of the method. Several characteristics make this procedure very attractive as a diagnostic option for white tail disease. The assay can be performed in most

laboratories; it requires no specialized equipment, a large number of samples can be examined in a micro titre plate at one time, a visual interpretation of positive or negative reaction is possible with the naked eye and the test provides results in a few hours. This method could be used for epidemiological studies of the disease. The test has high specificity and sensitivity as they react to dilutions as high as 10μg mL^{-1} of total protein of infected prawn sample. Although specific to *Mr*NV the drawback of this method is the limited stock of antibodies, unless they are produced by hybridoma technology.

TAS-ELISA

Mouse monoclonal antibodies against *Mr*NV was prepared and used to develop a TAS-ELISA (Triple antibody sandwich enzyme-linked immunosorbent assay) for *Mr*NV detection (Qian *et al.*, 2006). This method was first developed by Thottappilly *et al.* (1998). Monoclonal antibody was produced by hybridoma technology. Polyclonal antibodies were produced by immunization of a New Zealand rabbit with purified virus emulsified with an equal volume of Freund's complete adjuant. The studies show the sensitivity of *Mr*NV detection with TAS-ELISA to be 0.98 ng for purified *Mr*NV and 1.2 μg mL^{-1} total protein for infected prawn samples. It is almost 5 times more sensitive compared with the reaction of S-ELISA by polyclonal antibody.

3. Genome based detection methods

Molecular detection methods provide specific and sensitive methods of virus detection.

Dot blot hybridization

Dot-blot hybridization, a method currently used for disease diagnosis in aquaculture, involves binding and detection of labeled nucleotide probe to specific sequence in a genomic DNA or mixture of nucleic acid. In order to determine the limit of this method in case of *Mr*NV RNA, dot-blot hybridization was first carried out using purified viral RNA (Sri Widada *et al.*, 2003). The method was then applied to total RNA extract from tissues of field samples. The probe used for hybridization was a DNA fragment derived from RNA1 of *Mr*NV, cloned in pBluescript. It was labeled with digoxigenin (DIG)-labeled nucleotide by PCR. Dot blot hybridization appears to be more sensitive than TAS-ELISA. It allows many samples to be processed at one time on a single membrane and can be used in routine health monitoring in prawn hatcheries and in epidemiological studies. *Mr*NV could be detected even in animals, which did not exhibit clear signs of infection. The detection limit of dot blot hybridization is 7 fg of viral RNA (Sri Widada *et al.*, 2003). The negative response of adult animals may arise either from the fact that they were healthy or because the viral content was too low to be detected.

In situ hybridization

Sri Widada *et al.* (2003) performed *in situ* hybridization very successfully for the detection of *Mr*NV in infected prawn samples. The *Mr*NV infected cells display a dark purple precipitate in infected body parts. The use of *in situ* hybridization helps studies on viral localization in tissues. *In situ* hybridization has confirmed that viral infection is confined to the striated muscles. However, Sahul Hameed *et al.* (2004 b) have reported the occurrence of *Mr*NV and XSV in all positive tissues and organs of *M.rosenbergii*. Although *in situ* hybridization requires a high degree of technical skill and expensive equipment, it is appropriate for basic research.

RT- PCR

RT -PCR has been proven to be a highly sensitive and versatile diagnostic method

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RT- PCR

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among those presently available for detection of WTD (Sri Widada *et al.*, 2003; Sahul Hameed *et al.*, 2004a; Sri Widada *et al.*, 2004). It can be applied for routine health monitoring, early virus detection, studying virus-host interaction, detection of carriers and screening of brood stock (Sri Widada *et al.*, 2003; Sahul Hameed *et al.*, 2004a) It is a method used to amplify cDNA copies of RNA. RT-PCR method has been developed having a detection limit of 0.25 fg of total RNA (Sahul Hameed *et al.*, 2004a).

Real Time PCR

Recently, the relationship between these two viruses in infected prawns was studied by real time PCR (Zhang *et al.*, 2006). This method can detect <10 copies of virus per reaction (25µl) and was much sensitive than conventional RT-PCR.

Multiplex RT-PCR

Yoganandan *et al.* (2005) and Tripathy *et al.* (2006) developed a one-step multiplex RT-PCR assay for simultaneous detection of *Mr*NV and XSV by a single tube. They designed one pair of primer for *Mr*NV RNA_2 and XSV virus capsid protein gene and performed an RT-PCR with both the primer pairs. MRT-PCR had a detection limit of 1 fg of total RNA template for both viruses. MRT-PCR will be useful in studying virus–virus interactions in WTD.

RT –LAMP

All the genome based diagnostic methods are time consuming and expensive for field applications. A loop mediated isothermal amplification (LAMP), initially described by Notomi *et al.* (2000), was developed for rapid diagnosis of white tail disease in *M. rosenbergii* (Pillai *et al.*, 2006). A set of four primers, two outer and two inner, were designed for *Mr*NV detection. An additional pair of loop primers was also used in an accelerated LAMP reaction for detection of XSV. The method relies on auto cycling strand displacement DNA synthesis by *Bst* DNA polymerase large fragment, a DNA polymerase with high strand displacement activity. The LAMP reaction is highly suited for disease diagnosis in developing countries as amplification of DNA can be detected without the use of agarose gel electrophoresis, by the production of whitish precipitate of magnesium pyrophosphate as a by -product (Pillai *et al.*, 2006). There is no inhibition of amplification with increasing amounts of templates used. This is another advantage compared with RT-PCR, where a significant inhibition of amplification was observed when a greater quantity of template was used (Sri Widada *et al.*, 2003; Sahul hameed *et al.*, 2004a). As the reaction is carried out under isothermal conditions, it can be performed with a simple and inexpensive water bath. As there is no time loss in thermal changes, the amplification efficiency of the RT-LAMP method is extremely high. LAMP can amplify target nucleic acid to 10^9 copies at 60-65^{0}C in 1 h. (Notomi *et al.*, 2000; Parida *et al.*, 2004; Savan *et al.*, 2005). This method is more sensitive than RT-PCR for detecting *Mr*NV and XSV. 10-fold serial dilution of the positive samples showed that LAMP reaction without loop primers was 10 times more sensitive than RT-PCR for *Mr*NV detection and with loop primers 100 times more sensitive than RT-PCR for XSV detection. The rapidity and sensitivity of the LAMP reaction and more importantly, its simplicity of use, make it a highly suitable and cost-effective health management tool in shrimp and prawn culture (Pillai *et al.*, 2006).

Conclusion

To control the spread of viral diseases such as WTD, brood stock and seed screening should be strongly encouraged. The brood stock and seed infected with virus must be discarded with proper zoosanitory

methods. Usual sanitation and control protocols for viral infections are recommended. Water sedimentation and strict disinfection of tanks, water and brood prawn proved to be useful for disease control in affected regions.

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