GIANT FRESHWATER PRAWN, *MACROBRACHIUM ROSENBERGII* (DE MAN 1879): A REVIEW

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Received: September 22, 2018 Accepted: November 04, 2018

ABSTRACT*: In India, 11 species of shrimp have been found to be suitable for culture among 23 species available. Litopenaeus vannamei, Penaeus monodonand Macrobrachium rosenbergii are the major three species contributing great economy in Indian aquaculture industry.Total freshwater aquaculture production increased in 2016 compared to previous year.Aquaculture production of M. rosenbergii increased from 8,729 tonnes in 2015 to 10,152 tonnes in 2016. Giant freshwater prawn M. rosenbergii is becoming an important targeted species, as its culture is considered to be the potential source of income among farmers. This study reviews the current production status of freshwater prawn, white tail diseaseand its diagnostic methods and potential drugs available to treat the prawn diseases.*

Key Words: *Giant freshwater prawn, Macrobrachium rosenbergii, aquaculture, Diseases, Diagnostic methods, Immunostimulants.*

Introduction

Aquaculture, the practice of growing freshwater and marine plants and animals like finfish and shellfish under human-controlled conditionsis not a new concept. The term 'Aquatic' refers to a variety of water environments such as freshwater, brackish and marine. Freshwater culture is the cultivation of aquatic organisms where the end product is raised in freshwater, such as reservoirs, rivers, lakes, canals, ponds and groundwater, in which the salinity does not normally exceed 0.55%. In India, many crustacean species are cultured, the predominant commercial species being brackish water shrimps, freshwater prawns, and freshwater/brackish water crabs. The giant freshwater prawn scientifically known as*Macrobrachium rosenbergii* (popularly known as 'scampi') is one of the major cultivable aquatic animals contributing in Indian aquaculture system. *M. rosenbergii* is also known as the giant river prawn or the Malaysian prawn. *M. rosenbergii* is a native prawn of Thailand and other Southeast Asian countries including Vietnam, Cambodia, Malaysia, Myanmar, Bangladesh, India, Sri Lanka and Philippines. It was the pioneering works of Shao Wen Ling (1961) and Tukuji Fijumura (1972) that made possible commercial development of freshwater prawn culture (New, 2000) and hence they are considered as fathers of freshwater prawn farming.In India, farming of *M. rosenbergii* was felt as an alternative to the tiger shrimp,*P. monodon*, the farming of which had suffered heavily due to viral infections (Sahul Hameed *et al.,* 2000).This study reviews the current production status of freshwater prawn, white tail disease and its diagnostic methods and potential drugs available to treat the prawn diseases.

Giant freshwater prawn, *M. rosenbergii* as the candidate species for freshwater aquaculture in India.

All the freshwater prawns that have been cultured belong to the genus *Macrobrachium*, the largest genus of the family *Palaemonidae,* which contains more than 200 species, almost all prawns completing at least a portion of their life cycle in sea water (New, 2002). The first commercial giant freshwater prawn hatchery in India was established at Bhimavaram of Andhra Pradesh in 1990; but freshwater prawn farming assumed national significance only after a decade (Anon 2001, 2003; Nagarajan and Chandrasekhar 2002). *M. rosenbergii* has many advantages such as fast growth rate, compatibility for poly-culture, high protein content, disease resistance, merges perfectly with freshwater ecosystem, lesser man power is required, management measures are minimal (Murthy, 1998).Freshwater prawn farming supports and supplements the paddy cultivation, enhances productivity and income, waste production is minimal when compared to the marine shrimp farming and no adverse ecological impact.

Global Production Status of *M. rosenbergii*

Thailand and Vietnam first reported giant freshwater prawn production to FAO in 1975, followed by Myanmar (1977), Taiwan (1982), India (1989), Bangladesh (1995) and China (1996). It is encouraging to see that some relatively new countries with considerable rearing and marketing potential are beginning to

report production of giant freshwater prawns, notably Iran since 2001, Indonesia (2002), the Philippines (2005), Sri Lanka (2007) and Cambodia (2008). According to recent reports, giant freshwater prawn, is now considered to be one of the most important crustacean speciescontributing to the global prawn aquaculture industry and great interest is now focused onimproving the productivity of this species (Nhan*et al*., 2009; Thanh*et al*.,2009, 2010; Aflalo*et al*., 2012; Kitcharoen*et al*., 2012; Luan *et al*., 2012). The major giant freshwater prawn producing countries in Asia are Bangladesh, China, India, Myanmar, Thailand and Vietnam. The total freshwater prawn production of India in 2016 was 10,152Metric tonnes (MT) reported byMarie Product Export Development Authority of India(MPEDA). Production in Bangladesh is currently running at 46,189MT in 2016. Annual production in China is 1,32,278MT. Production in Myanmar is currently running at nearly 13,544.77 MT in 2016 (MPEDA). Production of freshwater prawns in Thailand increased from a meagre quantity of less than 5 MTin 1976 to 9,917 MT in 2000. The production in Thailand is currently running at 14,950 MT in 2016.Vietnam currently has similar levels of annual production but while the production in Vietnam appears to be between 7000 and 8000 tonnes (7,014 MT in 2016). In Malaysia, production expanded very significantly in 2016 compared to 2015. Malaysian output of farmed giant river prawns has oscillated quite wildly over the years and production in 2016was309.43MT.Production of Indonesia was increased to 2016 is 11, 708 MT (MPEDA). Compared to Asia, the production of giant freshwater prawns in other continents is very low. Output from the America, Brazil Cambodia, Iran, Peru and Sri Lanka has failed to expand. Both Ecuador and Mexico produced several hundred Metric tons in 1980 but reported little or no production in 2016. Production of *M. rosenbergii* in Brazil is on the decline and is currently about 100 MT. There is a great deal of interest in the seasonal production of *M. rosenbergii* in the temperate regions of the USA and there are considerable research activities there. The production *M. rosenbergii*in USA is 100 MT. Top 10 producers in 2016 are shown in **Table 1**and Global production status of farmed giant freshwater prawn, *M. rosenbergii* is given in **Table 2.**

Table 1. Top 10 countries production of *Macrobrachium rosenbergii* in 2016 (MPEDA).

S. No.	Country	Farmed production in MT
1.	China	1,32,278
2.	Bangladesh	46,189
3.	Thailand	14,950
4.	Myanmar	13,545
5.	Indonesia	11,708
6.	India	10,152
7.	Vietnam	7014
8.	Taiwan	6,437
9.	Philippines	1,299
10.	Malaysia	309

Table 2 Global production status of *M. rosenbergii* from 2010 to 2016 (MPEDA).

Country	Giant freshwater prawn, *Macrobrachium rosenbergii* production in year (MT)						
	2010	2011	2012	2013	2014	2015	2016
China	1,25,203	1,22,933	1,24,713	1,17,402	1,27,204	1,29,452	1,32,678
Bangladesh	30,636	39,868	45,162	43,713	45,167	42,523	46,189
Thailand	25,606	21,079.9	18,702	18,168	18,000	16,218	14,950
Indonesia	10,725	10,145	13,775	13,773	13,609	14,122	11,708
Taiwan	6,318	6,460	6,759	6,774	8,557	6,580	6,437
India	6,568	3,721	4,269	-	3,545	7,989	10,152
Vietnam	4,246	5,813	5,885	4,785	5,674	7,014	7,014
Myanmar	2,881	4,233	4,355	872	800	2,329	13,545
Philippines	1,418	1,625	1,487	1,676	1,682	1,486	1,299
Malaysia	619	334	413.28	457	398	268	390
Brazil	100	100	100	100	100	100	100
Others	396	657	603	461	402	380	318
Total	**2,14,716**	**2,16,968**	**2,26,203**	**2,08,180**	**2,25,138**	**2,28,960**	**2,44,780**

Production status in India

Freshwater prawn farming is a rapidly growing sector mainly due to the recent development of culture technologies and their greater environmental sustainability compared to other crustaceans, because of its large size, tolerance to changes in water quality, ability to cope with handling stress and ability to feed on unconventional feed (El-Sayed, 1997). Since 1999, the area under scampi culture has expanded

considerably and it has reached more than 4 million hectare and is still expanding in the various states of India. Production of giant freshwater prawn was 8729 MT in 2015, whereas, it increased to 10,152 MT in 2016 (MPEDA**).** West Bengal is ranked first among the states with a production of 3780 tonnes in 2016, Maharashtra ranked second with Metric 2,002 tonnes, Odisha ranked third with 1,504 MT and Gujarat ranked fourth with 1,310MT while, Tamil Nadu was ranked seventh with 86MT.State wise production detail of *M. rosenbergii* in India is shown in **Table 3.**

Table 3 State-wise production details of Scampi in India (MPEDA, 2015-2016).

Sl. No	State	2015-16
1	West Bengal	3,780
2	Maharashtra	2,002
3	Odisha	1,504
4	Gujarat	1,310
5	Andhra Pradesh	1,207
6	Kerala	263
7	Tamil Nadu	86
8	Goa	0
9	Karnataka	0
	Total	**10,152**

Problems in *M. rosenbergii* culture

Giant Freshwater prawn, *M. rosenbergii* is one of the most economically important farmed species in the world. Freshwater prawns are considered relatively less susceptible to diseases (Pillai and Bonami 2012). Freshwater prawn culture is rapidly increasing important species cultured in many prawn farming countries. However, one of the major problems is the high rate of mortality. Higher rates of stocking, small sizes at stocking, algal blooms, oxygen depletion and artificial feed polluted the pond water and caused mortality. Strain and stress to female prawn during breeding is another reason for poor growth and loss in numbers. When the culture period is prolonged, the older prawns get coated with an algal scum. Apart from these, prawns are affected by viral, bacterial, fungal, Protozoan, Metazoan and parasitic pathogens.

Diseases of *M. rosenbergii*

Incidence of diseases has increased in parallel with culture of scampi. Although the causes of most of the diseases of scampi are known, some of the diseases are still of unknown and hence considered as idiopathic diseases. Almost all the main groups of pathogens, namely, viruses, bacteria, fungi, yeast and protists have been reported in scampi. Among these pathogens, viruses constitute the most important group in terms of their impact on culture due to their pathogenicity, lethality and infectiousness (Pillai and Bonami 2012). White tail disease(WTD)of *M. rosenbergii* is the most important disease recorded to date and also the most well studied among the diseases of freshwater prawns. All the reported diseases of prawn are listed in the **Table 4.**

Viral Diseases

Macrobrachium Hepatopancreatic Parvovirus (MHPV), white tail disease (WTD) and *Macrobrachium* nipponensis Reovirus (MnRV) are viral diseases with freshwater prawn as the principal host. Other viral diseases, such as Infectious Hypodermal Haematopoietic Necrosis Virus (IHHNV), White Spot Syndrome Virus (WSSV) and Monodon Baculo Virus (MBV) infecting Penaeid shrimp have been reported in Scampi. However, except for WTD, no other viral disease has made a serious impact on scampi production.

Table 4 Table showing the diseases of freshwater prawn. *M. rosenbergii*

VIRAL DISEASES	BACTERIAL DISEASES
Macrobrachium hepatopancreatic parvovirus (MHPV)	Larval Mid Cycle Disease (MCD)
Macrobrachium nipponensis Reovirus (MnRV)	Luminescence Disease
White Tail Disease (WTD) (*Mr*NV/XSV)	Bacterial Necrosis
Infectious hypodermal and haematopoietic necrosis virus (IHHNV)	Exuvia Entrapment Disease (EED)
Monodon baculovirus (MBV) infection	Shell Disease

White Spot Syndrome Virus (WSSV)	White post larvae Disease
OTHERS DISEASES	
Idiopathic Muscle Necrosis (IMN)	Black Gill Disease
Branchiostegite Shell Disease	White Prawn Disease (WPD)
Red discoloration Disease	Black Spot Disease
Brown spot disease	Parasitic Diseases
Appendage Deformity Syndrome (ADS)	

***M. rosenbergii* Nodavirus and Extra Small Virus (*Mr*NV/XSV)**

White tail disease (WTD) causes immense economic loss due to high mortality of the post-larvae in the hatcheries and nurseries (Arcier *et al.,* 1999). The disease was first reported in the French West Indies (Arcier *et al.,* 1999), later in China (Qian *et al.,* 2003), India (Sahul Hameed *et al.,* 2004), Chinese Taipei (Wang and Chang 2006), Thailand (Yoganandhan *et al*., 2006), Australia (Owens *et al.,* 2009) and Malaysia (Saedi*et al.,* 2012). The occurrence of WTD is still being reported from all prawn growing countries. Causative organisms for WTD were found to be two viral pathogens namely *M. rosenbergii* nodavirus (*Mr*NV) and extra small virus (XSV). These viral pathogens are responsible for severe mortality in hatcheries and nursery ponds.

***Macrobrachium* Hepatopancreatic Parvo-Like Virus (MHPV)**

The first reported virus in *M. rosenbergii* was *Macrobrachium* hepatopancreatic parvo-like virus (MHPV). MHPV develops in the digestive tract, in the epithelial cells of hepatopancreas and midgut (Anderson *et al.,* 1990). The histopathological changes include hypertrophied Feulgen positive nuclei with chromatin margination. 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.Gangnonngiw *et al.,* (2009) reported the presence of unusual spherical to ovoid inclusions in nuclei of hepatopancreas tubule epithelial cells of *M. rosenbergii* and the results of PCR, *in situ* hybridization and Immunohistochemical tests for shrimp parvo viruses were found to be negative for previously reported MHPV. Results suggested a new type of parvo-like virus, not previously reported from *M. rosenbergii*.

Macrobrachium nipponensis Reovirus (MnRV)

*Macrobrachium nipponensis*Reovirus (MnRV) belonging to Reoviridae is a specific digestive disease. It was isolated in China (Hubei province) in the summer of 2008. Mortality in farms was variable, ranging from 15% to 60%. Infection developed in hepatopancreas and infected cells exhibited eosinophilic to pale basophilic inclusion bodies in the cytoplasm, about 10 µm in diameter. These inclusions were surrounded by a larger clear vacuole. The viral genomes contained 10 dsRNA segments (Shu, Unpublished data).

Infectious Hypodermal and Haematopoietic Necrosis Virus (IHHNV)

Infectious hypodermal and haematopoietic necrosis virus (IHHNV) was reported in *M. rosenbergii*. High mortalities (up to 100%) were encountered in post-larvae of *M. rosenbergii* in Southern Taiwan (Hsieh *et al.,* 2006). The virus is non enveloped, icosahedral in shape with 22 nm diameter, has a density of 1.405 g mL^{-1} in CsCl. IHHNV has a capsid made of four polypeptides of 37.5, 39, 47 and 74 kDa and its nucleic acid is linear ssDNA with 4075 nucleotides. The virus can be detected in histology by CowdrytypeA inclusion bodies. The infection was further confirmed by *in situ* hybridization using a DIG-labelled probe specific to IHHNV. Dot blot hybridization and PCR methods are also available for detection of the virus.

Monodon baculovirus (MBV)

MBV type virus belonging to Family Baculoviridae. It is a non-specific digestive disease. Presence of characteristic rounded occlusion bodies in histology and squash preparations makes it easily identifiable. Occurrence of MBV was reported in hepatopancreatic tubule epithelial cells of post-larvae of the giant freshwater prawn from Thailand. The inclusions resembled those produced by some Baculo viruses prior to formation of occlusion bodies that enclose virions in a poly hedrin protein matrix. The electron microscopic observation showed that the intranuclear inclusions contained bacilliform, enveloped virions (327±29 nm X 87±12 nm) with evenly dense, linear nucleocapsids surrounded by trilaminar envelopes (Gangnonngiw *et al.,* 2010).

White Spot Syndrome Virus (WSSV)

WSSV is highly pathogenic not only to penaeid shrimp, but also to marine crabs, freshwater prawns and crabs (Cai*et al.,* 1995; Lo *et al.,* 1996; Wang *et al.,* 1998; Chen *et al.,*2000 and Sahul Hameed *et al.,* 2000, 2001, 2002). Size of the virions is 80–120 X 250–380 nm and that of nucleocapsid is 58–67 X 230–350 nm. Density is 1.18–1.25 g mL^{-1}. The nucleic acid consists of double stranded, circular, supercoiled DNA of ~305 kb. There are approximately 531 ORFs for 181 functional proteins. Transcriptional and translational

analysis 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 (d.p.i) but from 5 d.p.i onwards the gene was undetectable (Yoganandhan and Sahul Hameed 2007). In histology, it can be recognized by hypertrophied Feulgen positive nuclei in target tissues. Molecular diagnostic methods include hybridization, dot blot or in situ using cloned genomic probes, PCR including regular, nested PCR and direct or indirect in situ PCR (Pillai and Bonami 2012).

Bacterial Diseases

In *M. rosenbergii* a wide range of bacteria has been isolated from rearing water, eggs, larvae, post larvae and adults. Most of these genera are part of the normal micro flora of cultured crustaceans. These bacteria are common in water and grow-out pond. Opportunistic bacteria such as Vibrio spp., Aeromonas spp. and Pseudomonas spp. may cause major infections in freshwater prawn, *M. rosenbergii* just as in penaeids. Shell disease is known by severalnames such as brown spot, black spot, or burn spot. The cause of this disease is considered to be a multi factorial complex of epicuticle-cuticle damage or abnormality from mechanical, nutritional, chemical or other factors, followed by secondary bacterial or fungal infection. Infected prawns show progressive necrosis, inflammation and subsequent melanization on body and appendages. A variety of bacterial species, producing extracellular lipases or proteases such as *Aeromonas* sp., *Pseudomonas* sp., *Vibrio* sp., *Benekea* sp., have been implicated in shell disease. Shell disease, along with epibiont fouling, is one of the most common disease problems in cultured prawn (Sandifer and Smith 1985). Poor water quality and high organic load are associated with shell lesion-inducing bacteria (Cook and Lofton 1973). Exuvia entrapment disease affects early and late larval stages of *M. rosenbergii*. It is also known as metamorphosis moult mortality syndrome. Affected larvae are unable to free exuvia from appendages, eyes or rostrum in which they become entrapped. Luminescence disease caused by *V. harveyi* is very common in hatcheries of both freshwater and marine shrimps. The clinical sign of *V. harveyi* is luminescence, it is observed in during night time. Infected larvae also show fouling, opacity, slow swimming, aggregation and may reach 100% mortalities. Clinical signs of bacterial necrosis are a bluish colour or discoloration, empty stomach, weak larvae falling to the bottom of the tank, and brown spots on antennae and newly formed appendages. Mixed bacterial infections were observed, with filamentous *Leucothrix* spp. and non-filamentous bacilli and cocci present on the setae, gills and appendages (Tonguthai, 1992, 1997; Bueno and Gastelu, 1998). Enterococci are also implicated in clinical signs in juveniles and adults with yellow exoskeleton, swollen hepatopancreas, milky haemolymph and opaque and whitish musculature. Rickettsial disease causes whitish colouration of the larvae and is implicated in 'white post-larval disease' (Lacroix*et al.,* 1994). It mainly affects larval stage IV and V and can be treated with oxytetracycline or furazolidone (Da Silva *et al.,* 1989).

Fungal diseases

*Lagenedium*and *Fusarium* have been reported to cause mortalities in post-larval stages of *M. rosenbergii* in hatcheries (Gomes *et al.,* 1988; New, 2002). The mycelia invade the larvae and the mycelial network becomes very clearly visible under the cuticle of the larvae. The fungi *Fusarium solani* causes secondary infection in adults (Burns, *et al.,* 1979). The yeasts *Debaryomyceshansenii*and *Metschnikoviabicuspidata* cause infections in juveniles of freshwater prawn, *M. rosenbergii* that may manifest as yellowish, greyish or bluish muscles in juveniles (Chen 1995; Sung *et al.,* 1998).

Larvae are more susceptible than adults to infestation with protozoa. When protozoa are observed on the larvae, the water quality must be improved. *Corthunia* sp., *Epistylis* sp., and *Vorticella* sp., were the most common peritrichous ciliates in cultured prawns (Hall, 1979). The infestation sites are body, eye stalk, antenna, uropods and egg.*Zoothamnium* prefers the gills (Johnson, 1978). Prawns have an increased oxygen demand just prior to moulting and heavy fouling can be associated with mortality due to anoxia (Fisher, 1977).

Metazoan diseases

A few metazoan parasites such as*Digeneanmetacercariae* and parasitic isopods have been occasionally reported from *M. rosenbergii.*Digenean infections may have zoonotic importance in areas of where *Macrobrachium* is consumed raw. Velasquez (1975), for example, reported the presence of metacercariae of the microphallid*Carneophallusbrevicaeca*in the muscle of naturally infected *Macrobrachium* sp. from Philippines. These parasites have not been reported to cause disease in giant freshwater prawns and thus will not be considered further in the risk analysis.

Other Diseases

Idiopathic muscle necrosis (IMN) is known by various names such as white muscle disease, muscle necrosis, spontaneous muscle necrosis, muscle opacity or milky prawn disease (Akiyama *et al.,* 1982; Nash

et al., 1987; Brock, 1988). It causes massive larval mortalities in hatcheries. Nash *et al.,* (1987) reported that IMN caused sudden mortality of up to 60% in 28 day old post larvae in intensive rearing systems in Thailand. White prawn disease (WPD) is a disease of adult *Macrobrachium*, mainly in females. Johnson (1978) has reported whitening in *M. ohioni*adults in Texas. The subcuticular tissues appeared milky but the muscles were normal. No micro-organisms were demonstrated. Delves-Broughton and Poupard (1976) described a disease in *M. rosenbergii* and named it as white syndrome. It was characterized by a dense opaque white colour with soft skeleton. A reddish abdominal discoloration affecting adult prawns is often observed in rearing ponds, but the aetiology is unknown. Too much light, diet and stress have been considered to be responsible for this abnormality. Black gill disease is caused by precipitating chemical and nitrogenous waste products which are implicated in melanization of the gills (Johnson, 1982). Black spot disease is usually found focally on the gills, carapace, appendages, uropods, body cuticle and telson. They are self-limiting and shed with the exuvia in healthy prawns. In severe case of infection, it may spread to the epithelium, muscle and viscera, resulting in septicaemia and mortalities (Brock, 1983, 1988).

White Tail Disease (WTD)

White tail disease (WTD) is an important viral infection for *M. rosenbergii* due to large scale mortalities in hatcheries, leading to subsequent production losses in many countries such as Taiwan, Thailand, France, India and People's Republic of China (Bonami *et al.,* 2011). Since 1994, mortalities were episodically reported in a hatchery in Pointe Noire in Guadeloupe (French West Indies) (Arcier *et al.,* 1999).In India, these viral pathogens have been reported in hatcheries and nursery ponds located in Andhra Pradesh and Tamil Nadu (India) (Sahul Hameed *et al.* 2004).White tail disease was also reported in China (Qian *et al.,* 2003), Chinese Taipei (Wang and Chang 2006), Thailand (Yoganandhan *et al*., 2006), Australia (Owens *et al.,* 2009) and Malaysia (Saedi*et al.,* 2012). The occurrence of WTD is still being reported from all prawn culturing countries.

Clinical signs and histopathology of WTD

The main sign gave the name to this disease. The disease affects hatchery-reared larvae and postlarvae as well as nursery-reared early juveniles. The typical gross signs of WTD in infected post larvae were whitish coloration of muscles, starting in some areas of the tail, extending to the tail muscles (abdomen) and at a final stage to all the muscles of the prawn, comprising head (cephalothorax) muscles causing lethargy, abnormal behaviour, anorexia and weakening of their feeding and swimming ability. In all cases, mortality reached 100% within 2 to 3 days after the first appearance of prawn with whitish muscles (Arcier *et al.,* 1999; Sahul Hameed *et al.,* 2004; Sudhakaran *et al.,* 2007). When investigated by histology, lesions were evidenced essentially in muscle and connective tissues. There are basophilic cytoplasmic inclusions with a diameter of 1-40 µm in striated muscles of the abdomen, cephalothorax, and intratubular connective tissue of the hepatopancreas. Viral inclusions were not observed in epithelial cells of the hepatopancreatic tubules or in midgut mucosal epithelial cells was reported by Arcier *et al.,* 1999.

Causative organisms

The causative organisms for WTD are *Macrobrachium rosenbergii* nodavirus (*Mr*NV) and Extra Small Virus-like Particle (XSV). These two viruses developing in the cytoplasm were separated, purified and subsequently characterized (Bonami *et al.,* 2005). *Mr*NV is a small virus with icosahedral shape and non-enveloped particle (26-27 nm in diameter). It contains two single-stranded RNAs (RNA1-2.9 kb and RNA2-1.26 kb). *Mr*NV capsid contains a single amino acidchain of 43 kDa. Later, a second virus-like particle, unusually small, 15 nm in diameter and consequently named XSV (extra small virus-like particles), was also found associated with *Mr*NV (Qian *et al.,* 2003). XSV is also a non-enveloped icosahedral virus with a linear ssRNA genome of 0.9 kb encoding two overlapping structural proteins of 16 and 17 kDa (Sri Widada and Bonami, 2004; Bonami *et al.,* 2005). Because of its small size and absence of gene-encoding enzymes required for replication, it has been suggested that it is a satellite virus or helper virus for *Mr*NV.

Genome of *Mr*NV and XSV

The nodaviruses are known to contain a genome consisting of two single-stranded positive-sense RNA segments. RNA1, which encodes the viral part of the RNA-dependent RNA polymerase (RdRp) and RNA2, which encodes the capsid protein gene of 43 kDa (Bonami *et al.,* 2005). Sequence characterization of this RNA species showed that it is a subgenomic part of RNA-1, corresponding to its 30 region (Shi, unpublished data).Genome of XSV consists of a linear single-stranded positive-sense RNA coding for a capsid protein gene of 17 kDa (Capsid Protein-17). Because of small size and absence of gene, the encoding enzymes is required for replication, it has been suggested that XSV may be a helper virus, while *Mr*NV plays the role was reported by Sri Widada and Bonami (2004). Nucleotide sequencing of the *Mr*NV genome indicated that RNA-1 was composed of 3.2 kb nucleotides (GenBank Accession No. AY222839) and that RNA-2 contained 1.17 kb nucleotides (GenBank Accession No. AY222840). Both RNAs appeared to be in

sense orientation, thus allowing their direct translation. Partial amino-acid sequencing of the N-terminal end of Capsid Protein-43 established that this structural protein was encoded by RNA-2 and its estimated size corresponds to that of Capsid Protein-43 (Bonami *et al.,* 2011). Nucleotide sequencing of the XSV genome showed that the viral RNA is composed of 796 nucleotides and in sense 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) (Sri Widada and Bonami 2004). The XSV genome contained the coding sequence of the capsid protein Capsid Protein -17 or Capsid Protein -16 and no other coding sequences were found (Bonami *et al.,* 2011).

Diagnostic tools for White Tail Disease of giant prawn

Due to the importance of *M. rosenbergii* aquaculture and world trade, the availability of easy and rapid methods that also allow early diagnosis, is essential for routine monitoring of the animal health status and to restrain further disease outbreaks. Several diagnostic methods have been developed in different laboratories throughout the world to detect *Mr*NV and XSV, including histopathology, immunological methods, reverse transcriptase polymerase chain reaction technique (RT-PCR), loop-mediated isothermal amplification (LAMP), in-situ hybridization method, dot blot hybridization and high resolution melt (HRM) analysis for duplex detection of *M. rosenbergii* nodavirus.

ELISA

A sandwich enzyme-linked immunosorbent assay (S-ELISA) was developed for the detection of *Mr*NV using polyclonal antibodies produced in mice (Romestand and Bonami 2003). An antibody-coated plate was used to trap viral antigens present in the sample. The method appeared to be rapid, relatively inexpensive and exhibited high specificity. Triple antibody enzyme-linked immunosorbent assay (TAS–ELISA) was developed and used in virus diagnosis in post larvae hatcheries in China during the period 2000–2004 (Qian *et al.,* 2006). Plates were coated with rabbit polyclonal antibody to trap viral antigens.

Hybridization

Dot-blot hybridization was performed, using DIG-labeled and cloned parts of the *Mr*NV and XSV genome (Sri Widada *et al.,* 2003; Sri Widada and Bonami 2004). The methodology could be easily used to establish health status or *Mr*NV infection in samples of *M. rosenbergii*. The application out on field samples showed that the dot-blot hybridization can be carried on the RNA content of 14– 140 µg of tissue of diseased animals. The sensitivity of the method was established using successive 10-fold dilutions of total RNA extracted from an infected post larvae. In situ hybridization, DIG-labeled probes used were cloned parts of the viral genome. It appeared that *Mr*NV was found confined to the striated muscle and connective tissue of the abdomen, cephalothorax and appendages (Bonami *et al.,* 2011).

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

The genome of *Mr*NV and XSV is composed of linear ss-RNA. This ss- RNAs are prone to degradations and its amplification requires a prior step of reverse transcription (RT) using reverse transcriptase enzyme. Detection thresholds were determined by using successive 10- fold dilutions of viral RNA template. It appeared that RT-PCR was more sensitive than the dot-blot hybridization. In routine analyses, the RNA content of 0.15–1.50 mg of tissue is sufficient for RT-PCR. RT-PCR also allowed to detect the presence of *Mr*NV in samples showing no or very slight clinical signs (Sri Widada *et al.,* 2003; Sri Widada and Bonami, 2004). Different diagnostic methods have been used for detection of this disease but reverse transcriptase polymerase chain reaction (RT-PCR) technique is the most sensitive diagnostic tool among those presently available for detection of *Mr*NV. Early detection of viral pathogen would help in taking proper management steps to control the incidence of white tail disease in *M. rosenbergii* (Behera *et al.,* 2011 and Sahul Hameed *et al.,* 2011).

In situ RT-PCR

In situ RT-PCR was also developed using RT-PCR methodology and applied on microtome sections of tissue. Only 2–3 cycles of amplification are sufficient and amplifications are carried out using DIG labeled dUTP. Positive amplifications are then revealed by anti-DIG antibodies, according to the protocol used in in situ hybridization (Bonami *et al.,* 2011).

Real Time PCR

Real-time PCR is a diagnostic technique requiring technical skill and expensive equipment. It enables pathogen quantification in the infected tissue samples. The conditions of amplifications are similar to those used in RT-PCR. Also, the same primers can be used in RT-PCR, thus allowing control of the specificity of the amplification process. Control analysis of amplification is performed by electrophoresis on agarose gel. Real-time RT-PCR was successfully applied for *Mr*NV analysis in an in vitro viral development study using a cell line. This methodology can also be used in virus quantification in different tissues of

diseased animals to establish the infection mechanism of WTD (Chappe-Bonnichon, 2006; Hernandez-Herrera *et al.,* 2007).

RT-LAMP

Two sets of primers have been designed for each of the WTD pathogens. The two sets work well for specific *Mr*NV detection so long as the loop primers are not included as they induce self-amplification of primers in absence of any template. The two sets of primers of XSV specifically work well but only one set gave satisfactory results when the loop primers are included. Comparison with amplification by RT-PCR clearly showed that RT-LAMP is more sensitive. Without the loop primers, RT-LAMP is ten-times more sensitive and when the loop primers are included in the reaction, the sensitivity is much higher and is up to at least 1000 times more sensitive (Pillai *et al.,* 2006). RT-LAMP methodology does not require expensive equipment such as PCR machine and a simple water-bath is sufficient as the reaction is isothermal. RT-LAMP is a rapid and sensitive diagnostic tool for the detection of white tail disease of *M. rosenbergii*. This technique is "farmer friendly" as the detection can be carried out in any simple field laboratory. Visualisation of the result is convincing to the farmer.

HRM multiplex RT-PCR

HRM analysis is based on the determination of changes in fluorescence as a result of melting double stranded PCR products in response to 0.05 °C steps of increasing temperature. The melting temperature (Tm) and the characteristic shape in the melting curve profile of amplified products are highly dependent on nucleotide sequence (Senapin *et al.,* 2010). HRM technology was more sensitive and could be carried out faster than those employing nested RT-PCR followed by electrophoresis. HRM also offers the possibility of detecting sequence variation of DNA in the amplified products and reduced risk of amplicon contamination due to its single-tube reaction (Senapin *et al.,* 2010).

Immunostimulants for giant prawn, *M. rosenbergii*

Immunostimulants are chemicals, drugs and stressors that elevate the non-specific defense mechanisms or specific immune response. Immunostimulants activate the immune system of aquatic animals and render them more resistant to infection by viruses, fungi, bacteria and parasites. The use of immunostimulants for boosting the defense mechanism in crustaceans in general and prawns in particular is a new and promising field (Sung *et al*., 1994). The invertebrate possess very primitive defense system and therefore nonspecific immune system plays a vital role in prawns. One of the promising ways of strengthening the non-specific defense mechanism and protection of shrimp against diseases is by the administration of immunostimulants.

Beta (β) glucan

The β glucans are present in the cell wall of mushroom and yeasts. Glucan appear to show the most promise among all immunostimulants so far examined in fish and shrimp. Glucan extracted from *Saccharomyces cerevisiae*is one such type and is an important structural element of the yeast cellwall. β-glucan from yeast and fungi is classified as a potential immunostimulant that effectively triggers the immune systems of both vertebrates and invertebrates in several ways (Soltanian*et al.,* 2009). Anas*et al.,* (2003) reported that the combination of bacterins and yeast glucans gave better results as evidenced by 37 % increased post larval production of freshwater prawn, *M. rosenbergii.* In recent experiments, feed supplemented with β-glucan was also found to significantly enhance the expression of *M. rosenbergii*pacifastin heavy chain (*Mr*-PHC) in adult prawns, although this response was notably enhanced compared with stimulation by a pathogenic bacterium. Unfortunately, the mechanisms to explain these immunological responses are still unclear.

Lipopolysaccharides

Lipopolysaccharides (LPS) are located in the cell wall of gram negative bacteria where it forms a complex with lipids and proteins. They represent the O^- antigens and endotoxins of these organisms. LPS is recognized as a good immunostimulant for mimicking by microorganisms in the initiation of the immune response (McKay and Jenkin 1970; Söderhäll and Hall 1984;Perazzolo and Barracco 1997; Iwanaga *et al.,* 1998; Sung *et al.,* 1998, 2000; Takahashi *et al.,* 2000: Iwanaga and Lee 2005). LPS from gram-negative bacteria could increase activation of the proPO system (Söderhäll and Hall 1984; Perazzolo and Barracco 1997; Sung *et al.,* 1998), phagocytic activity (McKay and Jenkin 1970; Sung *et al.,* 2000). Differentially enhanced expression genes were isolated and identified through suppression subtractive hybridization (SSH) in the hemoyctes of LPS-treated and untreated giant freshwater prawns, *M. rosenbergii* (Lu *et al.,* 2009).

Other immunostimulants

Yeh *et al.,* 2006 reported that a quantity as low as 0.9 and 1.2 mg^{-1} saponin affects respiratory proteins and the acid-base balance, and decreases the immune competence of *M. rosenbergii* by decreasing

its phenoloxidase activity. This might lead to increased susceptibility of *M. rosenbergii* to pathogens. *M. rosenbergii* fed a diet containing bovine lactoferrin (Lf) at 100 mg kg^{-1} for 7 days increased its immune ability by increasing phenoloxidase activity, total protein and agglutinin levels together with an increase in resistance against *A. hydrophila* and nitrite stress. Reduced mortality was reported compared to the control group when challenged with *A. hydrophila*. Hence, polysaccharides like PG, LPS, BKC, levamisole, saponin and Lf showed the positive effect of protecting giant freshwater prawn against various bacterial and viral diseases.

Herbal immunostimulants for *M. rosenbergii*

Various Indian medicinal plants have been screened for antiviral activity against WSSV and aqueous extract of *Cyanodon dactylon* showed strongest antiviral activity against WSSV (Balasubramanian *et al.,* 2007, 2008). Only few studies were done on herbal based immunostimulants in prawn. Increased proPO activity, nitric oxide levels, and superoxide anion production in contrast to decreased SOD in *M. rosenbergii* fed with 0.1% and 11.0% *W. somnifera* supplementation diets act as a promoter of the prawn immune system against *A. hydrophila* infection (Harikrishnan *et al.,* 2012). Similarly Prophenoloxidase, Super Oxide Dismutase, Total Haemocyte Count and Clotting time were found to be significantly higher in *Cynodon dactylon* injected prawn (Farook et al., 2015).Turmeric powder has the capacity to modulate the antimicrobial peptides, particularly crustin and lysozyme, of the gaint freshwater prawn, *M. rosenbergii* when challenged with *V. alginolyticus*. Turmeric extract into commercial feeds also enhanced survival rate of shrimps challenged with a bacterial pathogen. Protective effect of the turmeric against viral infection will be worth pursuing and the plant can be exploited by the shrimp aquaculture industry for prawn feed formulation (Alambra*et al.,* 2012). *Musa paradisiaca* peels are generally regarded as waste materials. However, they are under-utilized sources containing a broad range of biological activities, such as antibacterial, anti-allergic, antioxidant activity and many more.

Prawn vaccines

Various viral recombinant proteins have been studied so far as vaccines in different host systems with notable effects against pathogens (Moreno-de lasHeras*et al.,* 2009). A high degree of protection was induced in *Marsupenaeus japonicus* against WSSV by intramuscular injection of WSSV recombinant proteins, rVP26 and rVP28 (Namikoshi et al., 2004; Caipang*et al.,* 2008). Witteveldt*et al.,* (2004) observed that VP19 was effective in *P. monodon*. This has also been confirmed in crayfish, *Procambarusclarkii*Jha*et al.,* (2006). Vaseeharan*et al.,* (2006) noted protection in *P. monodon* following vaccination with VP292. Satoh *et al.,* (2009) noted that kuruma shrimp, *Marsupenaeus japonicus* orally vaccinated with VP26 and VP28 was protected when challenged by feeding but not when challenged by injection. Development of subunit vaccines based on *Mr*NV replication-related proteins would be feasible and desirable as these proteins might interact with virus inside the host or trigger the host defence. The role of recombinant RNA-dependent RNA polymerase (RdRp) protein of *Macrobrachium rosenbergii* nodavirus (*Mr*NV) inmodulating the immune response and in reducing *Mr*NV load in infected giant freshwater prawn, *M. rosenbergii* has been investigated (Sahoo *et al.,* 2012).The r-MCP protein injected adult prawn and r-MCP protein immersed *M. rosenbergii*post larvae showed good immune response compared to control group by analysinghaematological, immunological parameters and study the immune-related and HSP gene expressions with β actin as internal control in various organs and whole post larvae using RT-PCRand real-time PCR. The percentage survival of post-larvae treated with purified r-MCP protein and challenged with *Mr*NV and XSV was 76.03% on 15th day post infection 99.78% survival was recorded in normal group on 15th day post infection and 0% survival was recorded in virus alone infected group on 10th day post infection (Farook et al., 2014).*Mr*NV-CP-RNA-2- pVAX1 treated groups had the survivals of 60 and 80% in 20 and 40 days post vaccination respectively but the non-vaccinated *M. rosenbergii* group showed 100% mortality within 5 days and enhanced haematological parameters also increased in 20 and 40 days post vaccination groups respectively, when compared with the non-vaccinated groups was reported by Citarasu et al. 2018.

RNA interference (RNAi)

Bacterial expressed dsRNA to manage disease has been elucidated by several workers (Timmons and Fire 1998; Sarathi *et al.,* 2008; 2010). Since both *Mr*NV and XSV are found associated with WTD, we used dsRNA corresponding to genes of both viruses either individually or in combination as RNAi therapeutics. The potential of RNAi as a novel strategy to address WTD of *M. rosenbergii* is encouraging. Studies have shown the successful use of RNAi against *Mr*NV B2 gene of *Mr*NV in redclaw crayfish (Hayakijkosol and Owens 2012). Bacterially synthesized dsRNA specific to Capsid protein of Hepatopancreatic parvo virus showed positive effects in experimentally injected freshwater rice field crab,

Paratelphusa hydrodomous (Herbst). Bacterially synthesized dsRNA of capsid and B2 genes of *Mr*NV and capsid gene of XSV would be better candidates than applying *in vitro* synthesized dsRNA for RNAi therapy. Bacteria expressing dsRNA were inactivated and delivered orally along with the feed. *Mr*NV and XSV delivered orally through the inactivated bacteria expressed dsRNA and induced protection in *M. rosenbergii* against WTD. Highest RPS was obtained with a combination of dsRNA of *Mr*NV and XSV capsid genes followed by the dsRNA of *Mr*NV alone. RNAi developed against B2 gene can be of universal interest and find application against different geographical isolates of *Mr*NV and *Pv*NV as the region is highly conserved (Naveen Kumar *et al.,* 2013).

Conclusion

Freshwater prawn farming plays an important role in India, contributing to increased food production,higher economic growth and increased employment opportunities. The practice of prawn farming has offered opportunity to increase incomes for farmers. A range of public and private sector investments are needed to realize the potential for growth and expanding economic output in prawn farming sector. Further researchare needed in prawn production technology, hatchery operations and strictly maintain standard operating procedures. Disease free brood stock are required for successful operation of prawn hatcheries. Low-cost pellet feed industries would help to increase farmers profit margins. Research in seed and feed production areas need to be given due attention, considering existing technology, the transfer, adaption and development of new technology.

Acknowledgements

The authors thank the management of Islamiah College (Autonomous) for providing the facilities and documents to carry out this review work.

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