Monodon Baculovirus of Shrimp

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Abstract Among the viruses infecting penaeid shrimp, monodon-type baculovirus (MBV) otherwise known as *Penaeus monodon* singly enveloped nuclear polyhedrosis virus (PmSNPV), is one of the widely reported and well described viruses. It is a rod-shaped, enveloped, double-stranded DNA virus, and considered till recently, as the type A baculovirus. Besides MBV, two strains of SNPV are reported—plebejus baculovirus and bennettiae baculovirus. MBV was reported to be originated from Taiwan and has wide geographic distribution and is reported to be enzootic in wild penaeids of the Indo-Pacific coasts of Asia. The virus also has diverse host-range including a variety of cultured and captured shrimp species and freshwater prawn, *Macrobrachium rosenbergii*. MBV has been reported in all life stages of *P. monodon* with late larval, postlarval and young juvenile as the most susceptible stages/ages. However, MBV has not been documented in early larval stages. Although MBV has been reported to be tolerated well by shrimp, the infection has been attributed to decreased productivity. The target organs or tissues of MBV are the hepatopancreatic tubules and duct epithelium of postlarvae, juveniles and adults, and the anterior midgut epithelium of very young postlarvae. The prominent clinical sign of infection is the presence of multiple spherical inclusion bodies in the hepatopancreas and midgut epithelial cells. The major mode of transmission of the virus is horizontal through oral exposure to occlusion bodies, contaminated tissues or fomites. Minor morphometric

variation of the virus has been reported among different isolates. The rod-shaped enveloped virus particles range from 265–324 nm in length and 42–77 nm in diameter. Although complete genome sequence of MBV is not available, nucleic acid of MBV is circular, double-stranded DNA with a genome size ranging from 80 to 160 kbp. The virus codes for a 53 kDa major polyhedrin polypeptide and two minor 47 and 49 kDa polypeptides. A variety of diagnostic tools have been reported for this virus including real-time PCR and LAMP-based detection. Taxonomic position is still uncertain and International Committee on Taxonomy of Viruses lists MBV as a tentative species named PemoNPV in the genus *Nucleopolyhedrovirus*. However, according to the latest genomic information on the virus, it has been suggested to create a new group of non-occluded bacilliform viruses called nudiviruses with MBV as one of the members. The aim of the current work is to describe the knowledge on the status, distribution and host-range, pathology, transmission, virus structure and morphogenesis, genomic characteristics, diagnosis and the latest taxonomic position of MBV.

Keywords Penaeid shrimp · Viral disease · Monodon baculovirus · MBV

Introduction

Penaeid shrimp culture, from its experimental beginnings, has been transformed into one of the fastest growing industries in the world, in the last four decades. Early part of 1990s had witnessed a booming of the industry world over. In 1997, global annual farmed-shrimp production was 700,000 MT and subsequently, the production has reached 3.2 million MT [110]. However, in the process of this

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transformation (expansion and intensification) shrimp culture had faced serious problems due to major epizootics which caused appalling economic losses in shrimp growing countries. Majority of these epizootics have been caused by viruses such as Taura syndrome virus (TSV), yellow head virus (YHV), *white spot syndrome virus* (WSSV) etc., apart from other significant viruses reported from penaeid shrimp [51]. However, viruses belonging to the family Baculoviridae are significant among the penaeid shrimp viruses. In fact, a member of this family, *Baculovirus penaei* was the first penaeid shrimp virus reported [25].

Baculoviruses are rod-shaped, enveloped viruses with double-stranded DNA. Three baculoviruses have been reported from penaeid shrimp which infect hepatopancreatic duct and tubule epithelium and the adjacent part of anterior mid gut [26, 52, 82]. Two of these viruses (*Baculovirus penaei* (BP) and monodon-type baculovirus (MBV)) are occluded, i.e. virions become embedded in a proteinaceous occlusion body [87] and one (*Baculovirus midgut gland necrosis virus*- BMNV) is non-occluded [10]. Accordingly, BP and MBV are grouped under the subgroup A or type A and BMN as subgroup C or type C. For many years, WSSV was also considered as a non-occluded baculovirus and during that time penaeid baculoviruses were grouped into following four groups [51]:

1. BP-type viruses or *Penaeus vannamei* singly enveloped nuclear polyhedrosis virus (PvSNPV)
2. MBV viruses or *Penaeus monodon* SNPV (PmSNPV) which includes bennettiae baculovirus (BBV) and plebejus baculovirus (PBV)
3. Baculovirus midgut gland necrosis type (BMN) or *Penaeus japonicus* non-occluded baculovirus (PjNOB I)
4. White spot syndrome baculovirus type (WSBV) or *Penaeus monodon* non-occluded baculovirus (PmNOB II- type) which includes systemic ectodermal and mesodermal baculovirus (SEMBV), rod-shaped virus of *P. japonicus* (RV-PJ), hypodermal and hematopoietic necrosis baculovirus (HHNBV) and white spot baculovirus (WSBV) or WSSV.

However, subsequently, based on the molecular evidence provided by van Hulten et al. [99], WSSV has been reclassified into a new family, *Nimaviridae*, and the International Committee on Taxonomy of Viruses (ICTV) approved the family name and a new genus, *Whispovirus* has been erected to include WSSV complex [68]. Further, according to the latest report, based on sequence analysis and molecular phylogeny, occluded shrimp monodon baculovirus or *Penaeus monodon* nucleopolyhedrovirus belongs to the nudivirus group and not to baculoviruses. Accordingly, Wang and Jehle [106] proposed the virus to be assigned to the genus *Nudivirus* and to rename it

P. monodon nudivirus, *PmNV*. However, in this review we use the name MBV, as this is the most popular name by which the virus has been described in the literature.

***Penaeus monodon*-type Baculovirus (MBV)**

MBV is one of the widely reported and well described viral pathogens of shrimp. MBV was first identified in 1977 from Taiwanese shrimps [47, 56]. Since then it has been identified and reported from a variety of penaeid shrimp species from different parts of the world [3, 44, 50, 54, 58, 69, 70].

MBV had been implicated as the causative agent of the first major crisis caused by a disease in the history of penaeid shrimp culture. This epizootic has resulted in the collapse of the industry in Taiwan in 1988 [60]. However, subsequent analyses showed that environmental and management factors also had contributed to the collapse [33, 48]. Further, according to Lightner [51], YHV and/or a septic hepatopancreas necrosis due to a *Vibrio* sp. were likely to be the cause. Later, it was also discovered that MBV did not cause shrimp mortality if the rearing conditions were good [31]. Nevertheless, this incidence brought MBV under focus till the research priorities on shrimp viruses have shifted to epizootics caused by WSSV, TSV, YHV etc.

MBV is a rod-shaped, singly enveloped, occluded (in paracrystalline intranuclear occlusion bodies) baculovirus with double-stranded DNA which replicates within the nucleus. However, Mari et al. [67], based on the morphological studies, opined that considering the presence of characteristic appendages at each extremity of the enveloped particles, superficial helical structure and 2 layered 'caps' that enclose the extremities of the nucleocapsids, and the braided-like structure interpreted as nucleoproteins associated with nucleocapsids, MBV might be more closely related to the non-occluded crustacean baculoviruses than to the occluded-type A insect baculoviruses. Because of its single enveloped nature, Mari et al. [67] had given MBV from *Penaeus monodon* the designation PmSNPV (singly enveloped nuclear polyhedrosis virus from *P. monodon*). Three strains of SNPV are reported-MBV, PBV and BBV.

Geographic Distribution and Host-Range

Among the three strains of SNPV, MBV-type baculovirus has wide geographic distribution and diverse host-range. Although MBV was first reported from *P. monodon* imported from Taiwan to Mexico [52, 56] the presence of MBV and its wide distribution in Taiwanese shrimp culture area was demonstrated by Lightner et al. [57] and Chen

et al. [20] after an extensive survey. Later studies revealed close relation between MBV and losses due to diseases in many shrimp culture facilities in Taiwan and many culture areas of Indo-pacific region as far north as Japan [4–6, 20, 23, 24, 30, 31, 46, 59, 69–71, 94, 105]. Subsequently, surveys of shrimp culture facilities as well as wild shrimp have documented and reported wide spread distribution of MBV from different parts of the world [1, 32, 72, 80]. Among the geographical regions, wide distribution has been demonstrated in the Indo-pacific coasts of Asia and it has also been reported that MBV is enzootic in wild penaeids throughout this region [51]. Chanratchakool et al. [17] has observed that MBV may be very widespread in Southeast Asia. Further, it is reported that in Taiwan co-infection of MBV and WSSV is a common occurrence in *P. monodon* [107]. According to Lightner [51], the virus has presence in Indo-pacific countries (Peoples Republic of China, Taiwan, Philippines, Malaysia, Singapore, Thailand, Sri Lanka, India, Indonesia and Australia), in the Middle-East (Kuwait, Oman and Israel), Mediterranean (Italy), Africa (Kenya, Gambia), Pacific (Tahiti, Hawaii) and Americas (Mexico, Ecuador, Brazil, Puerto Rico and in several Southeastern US states). The virus has been recorded in cultured *P. monodon* and *P. indicus* in India by

Vijayan et al. [104]. Later, the virus has been reported from Vietnam [42, 75] and continued presence or new host species have been recorded from certain regions, especially from India, which have already reported the presence of MBV [1, 2, 45, 76, 80, 81, 88, 95–98, 100].

MBV has been reported from a variety of penaeid shrimp species cultured or captured or imported in wide geographical locations. Although wild *P. monodon* appeared to be the primary host of this virus, it has also been recorded from *P. penicillatus*, *P. indicus*, *P. semisulcatus*, *P. merguensis*, *P. kerathurus*, *P. esculentus*, *Litopenaeus vannamei*, *Metapenaeus ensis* and *M. lysianassa* (Table 1). Recently, the virus has been recorded from freshwater prawn, *Macrobrachium rosenbergii* [40]. A list of host species and various geographical locations where the virus has been recorded is summarized in Table 1.

MBV Disease: Clinical Signs, Symptoms, Histopathology, Pathogenesis and Control Measures

MBV has been observed in all life stages of *Penaeus monodon* and is the most common virus of *P. monodon*

Table 1 List of host species and geographic locations of MBV

Host species	Geographic location	References
<i>Penaeus monodon</i>	Mexico (host originated from Taiwan)	[52]
	Taiwan, Philippines, Tahiti	[56]
	French Polynesia	[57]
	Israel	[23, 24]
	Malaysia	[3]
	Indonesia	[69, 94]
	Australia	[30, 78, 101, 102]
	Thailand	[18, 31, 38, 92]
	Philippines	[6, 28, 71]
	India	[1, 2, 32, 45, 65, 76, 77, 80, 81, 88, 95–97, 100, 104]
<i>P. indicus</i>	Vietnam	[75]
	India	[104]
<i>P. penicillatus</i>	Vietnam	[42]
	Taiwan	[20, 57]
<i>P. merguensis</i>	Australia	[30]
<i>P. kerathurus</i>	?	[50]
<i>P. semisulcatus</i>	Israel	[23]
<i>Litopenaeus vannamei</i>	?	[51]
<i>Metapenaeus ensis</i>	Taiwan	[20]
	Vietnam	[42]
<i>M. lysianassa</i>	Vietnam	[42]
<i>M. monoceroros</i>	India	[66]
<i>M. elegans</i>	India	[66]
<i>Macrobrachium rosenbergii</i>	Thailand	[40]

? mark indicates location of collection is not known

with late larval, post-larval and young juvenile shrimp as the most susceptible stages/ages. However, early larval stages have not been documented with MBV disease [10] and no occlusion bodies (OB) could be detected in larvae before PL2 stage [20]. Further, Lightner et al. [56] reported decreased severity with increased size/age of shrimp in which exposure of MBV to larval and post-larval stages is likely to result in severe infection and mortality than the exposure to juveniles and adult stages. According to these authors, three stages in the pathogenesis could be noticed in the hepatopancreas: stage 1- hepatopancreatocytes with slightly hypertrophied nuclei containing a few completed virions but without any occlusions; stage 2- hypertrophied nuclei with developing occlusions and developed virions; and stage 3- hepatopancreatocytes with mature occlusions and abundant number of completed free and occluded virions. The last stage is followed by cell necrosis and cytolysis.

In the case of freshwater prawn, *Macrobrachium rosenbergii*, MBV infection has been reported in the postlarvae (PL10–PL23) [40]. MBV produces similar lesions in the hepatopancreas of *M. rosenbergii* as that of *P. monodon* at the early stage of infection. The hepatopancreatic tubule epithelial cells show enlarged nuclei with central eosinophilic occlusions and margined chromatin. However, except in some samples, occurrence of OB was reported [40]. Gangnonngiw et al. [40] have reported moderately electron dense, spherical particles resembling polyhedrin subunits of OB similar to MBV of tiger shrimp. Although based on the limited samples, these authors have suggested that occurrence of OB is rare in *M. rosenbergii*. Further, they also stated that unlike in *P. monodon*, diagnosis of infection by examining squash preparation of hepatopancreas stained with malachite green to detect the OB would not be reliable in the case of *M. rosenbergii*.

MBV infections have been reported to be well tolerated by shrimp if other culture conditions are optimal [31, 57]. The tolerance of *P. monodon* to MBV has also been reported by Flegel et al. [37]. Severe infection with high prevalence has been recorded in cultured shrimp with no serious impact on the health of shrimp [104]. However, Lightner et al. [56] reported that crowding and facultative pathogens could enhance the severity and prevalence in raceway-cultured *P. monodon*. On the contrary, Natividad and Lightner [72] reported that MBV infections would increase the susceptibility of shrimp to secondary infection. Absence of serious impact is due to the fact that disease remains latent until occurrence of a stress to the infected animal which leads to the development of disease. It has been recorded that MBV infected larvae of *P. monodon* and *P. penicillatus* and broodstock of *P. monodon* showed significant mortality due to transfer stress [20]. However, according to the report, a heavy bacterial infection was

noticed in the hepatopancreas of MBV-infected animals. Heavy mortality associated with MBV infection has been reported by Lin [60], Tangtongpiroj [90] and Thikiew [92]. Although pathogenicity of MBV has not been unequivocally established, as recorded by Fegan et al. [31], the opinion that MBV did not cause mortality was expressed by Liao et al. [48] also. Further, it has been reported that MBV does not cause high mortality, but is believed to lead to a decrease in productivity [36]. Subsequently, it has been recorded that *P. monodon* showing slow-growth syndrome had high prevalence of combined infection of HPV/MBV [18]. Further, the presence of MBV has been recorded as one of the non-lethal viral infection apparently tolerated by shrimp in disease outbreaks associated with other viruses such as YHV [35]. Considering all the reports, it is obvious that the MBV disease may lead to reduced growth, value of harvest and overall health of the animal, apart from pre-disposing the infected shrimp to other pathogens resulting in increased mortality. On the contrary, it is also suspected that poor rearing condition would flare up the virus followed by secondary infection resulting in mortality [34]. A recent study has reported that low stocking (6 PL/m²) density with MBV-infected seeds can be successfully cultured by proper water quality and feed management [41]. However, Flegel [34] had opined that although MBV is not a serious pathogen, it should be eliminated from the farming system because it is unlikely that shrimp will carry heavy infection without any ill effect.

Epizootiological study on MBV in Taiwan showed a tremendous increase in prevalence of the virus in postlarvae, juveniles and broodstock of *P. monodon* over a period of time [20]. According to the report, MBV infection rate in postlarvae showed an increase from 17 to 85 % in 4 years period, and almost similar trend was noticed in juveniles as well. Infection in wild broodstock of *P. monodon* during the study showed a prevalence of 52 %. However, postlarvae and juveniles of *P. penicillatus* recorded a prevalence of 30 and 28 %, respectively. Vijayan et al. [104] also reported a high prevalence of MBV in cultured (juveniles and adult stages) *P. monodon* and *P. indicus* from India (37.3 and 33.6 %, respectively).

Gross signs of MBV infection include lethargy, reduced feeding and preening activities and growth rate. It has also been reported that MBV infection is a pre-disposing factor for increased occurrence of gill and surface fouling organisms [104]. Similarly, severely affected shrimp appeared lethargic, anorexic and are predisposed to fouling and bacterial infection resulting in localized 'shell disease-type' lesions of the gills or cuticle or as bacterial septicemias [50]. Although Flegel et al. [37] reported that MBV infection did not show statistical correlation with stunted growth, it is reported that moderately to severely infected postlarvae of *P. monodon* appeared to be smaller

and darker than less or uninfected animals. Later, Flegel et al. [39] also reported that length of MBV-infected shrimp was significantly shorter than uninfected shrimp from the same pond. They noted that the difference in growth is not easily detected until the late phase of the cultivation cycle. Flegel et al. [39] also stated that slow growth of MBV-infected shrimp was reported from the intensive culture system in Philippines where it necessitated prolonging the culture period to obtain larger shrimp. Further, severely infected larvae and postlarvae show white midgut line through the abdomen. According to Lightner et al. [56], the target organs and tissues for MBV are the hepatopancreatic tubule and duct epithelium of postlarvae, juveniles and adults, and the anterior midgut epithelium of very young postlarvae. A prominent clinical sign of the infection is the presence of multiple, spherical OB in the hepatopancreas and anterior midgut epithelial cells. Histopathologically, infected cells exhibit necrosis, lysis and sloughing-off of cells into tubule lumen [6, 52, 104]. Ramasamy et al. [81] have reported a 300 % volume increase in the hypertrophied nuclei of the MBV-infected hepatopancreatocytes. Similar histological changes such as the presence of enlarged nuclei with diffuse, central, eosinophilic occlusions and marginated nucleoli and chromatin in the hepatopancreatic cells have been reported in *M. rosenbergii* as well [40]. Further, susceptibility of monolayer cultures derived from lymphoid organ of *P. monodon* to monodon baculovirus has been recorded by Chen and Kou [19].

Major mode of transmission of this virus is horizontal. The occlusion bodies, which are composed of protein matrix, polyhedrin that contain embedded viral particles, are the source of viral transmission from one animal to another. The OBs are released through faecal matter of infected broodstock as well as larvae, and these are ingested by other larvae. Accordingly, it has been reported that MBV is horizontally transmitted by oral exposure to free viruses and OBs, contaminated tissues or fomites. Although the virus can transmit vertically from infected mother to the offspring through contamination, transovarian transmission of infection has not been reported. According to Brock and Lightner [10], experimental horizontal transmission of MBV was not successful, and they have opined that this might be due to the inactivation of virions by freezing and slow thawing. However, Chen et al. [21] and Paynter et al. [78] have reported horizontal transmission of MBV by oral ingestion of occluded or free virions. Further, Paynter et al. [78] demonstrated that virus can be transmitted horizontally directly through water column.

Since the virus gets transmitted from infected individual shrimp through horizontal means, the best method of controlling the infection is by eradicating the infected

broodstock and following strict disinfection procedures. Various chemical agents have been reported to be effective in inactivating MBV such as formalin, chlorine and iodophore, and have been recommended for disinfecting shrimp hatcheries. Chen et al. [21] have tested various washing procedures to eliminate MBV infection in larval stages of *P. monodon* and reported that a combined washing with formalin followed by iodophore could eliminate the infection completely from naupli or fertilized eggs. However, it has been reported that MBV is more resistant to these chemical treatments than other shrimp viruses and, therefore, higher concentration of the chemicals are required to inactivate the virus [85]. Accordingly, Manisseri et al. [64] have reported that previously recommended dosage of iodine will not be sufficient to inactivate MBV, and they recommended that disinfecting solution used in hatchery should contain at least 10,000 ppm iodine for complete inactivation of MBV. Considering these facts, a practical approach in the removal of MBV should involve different methods such as eradication of infected stocks, strict disinfection regimes in the hatchery [9, 21, 111] using different chemicals at appropriate concentrations.

Virus Structure

Ultrastructure of the virions and OB has been well documented by Brock and Lightner [10] in a review. Morphogenesis of cellular membrane structure of MBV infected penaeid shrimp cells has been studied by Lu et al. [63]. However, the documentation was based only on morphological data derived from transmission electron microscopic studies of the infected cells. Later, MBV was successfully isolated from infected hepatopancreas of *P. monodon* using sucrose gradient ultracentrifugation by Mari et al. [67]. By collecting different fractions they could purify virions, envelopes, nucleocapsids and polyhedron occlusion body subunits. According to them intact virion particles measure 265–282 nm in length and 68–77 nm in diameter. Enveloped particles contain one capsid per envelope. Envelope surface is composed of small uniformly sized granular structure interspersed with small spikes which are prominent at the apex of the intact particle. PTA staining reveals the appendages at the extremity of the envelope which is believed to be envelope extensions. Nucleocapsids possess rows of surface structures, spaced about 6 nm apart and arranged in a helical pattern. These structures give the nucleocapsid surface a ‘cross-hatched’ appearance because of their position at an angle with the axis of the nucleocapsids. The non-enveloped nucleocapsids measure 250–269 nm in length and 62–68 nm in diameter. However, according to these

authors, some disrupted nucleocapsids show only one closed extremity and others did not show any cap-like structure. Further, they also recorded a protruding braided-like structure in the nucleocapsids which they interpreted as nucleoprotein. Ramasamy et al. [81] reported two types of MBV OB in *Penaeus monodon* of India, with Type 1 OB containing rod-shaped virions, occluded and non-occluded with a double-layered envelope, and the enveloped virions measuring $267 (\pm 2)$ nm long and $78 (\pm 3)$ nm wide with a nucleocapsid $54 (\pm 2)$ nm long and $161 (\pm 3)$ nm wide. Virions observed in the Type 2 OB possessed a single-layered envelope and measured $326 (\pm 4)$ nm long and $73 (\pm 1)$ nm wide with a nucleocapsid $295 (\pm 1)$ nm long and $67 (\pm 1)$ nm wide (Table 2). All the viruses placed under the genus nudivirus are rod-shaped and except for the *Heliothis zea* nudivirus-1 (HzNV-1), they all possess a protruding structure and which in the case of MBV is on the envelope surface. Although a tail-like appendage on the envelope surface was also recorded for the tentative crab nudivirus, as well as WSSV, functional significance or relationship of this structure is not known yet. Further resemblance in virion architecture, genome structure and DNA replication strategy has been reported among WSSV, nudiviruses and baculoviruses [99, 113]. Characteristic features of three PmSNPV strains are given in Table 2.

Mari et al. [67] also reported purified polyhedrin occlusion body subunits (OBS) of MBV which measure 22–23 nm in diameter resembling icosahedral virus particles. However, using transmission electron microscopy on the tissues section of infected hepatopancreas, Johnson and Lightner [44] reported the approximate size of OBS to be 18 nm while Chen et al. [20] and Boonsanongchokying et al. [8] reported a size of ~ 20 nm. OB subunits are generally rounded but some are six-sided. The six-sided profile of MBV OBS and the apparent cubic symmetry would explain how the OBS assumes a tridimensional crystalline structure [67]. MBV has one of the largest OBS among the 3 virus families reported to exhibit polyhedrins

such as *Baculoviridae*, *Reoviridae* and *Poxviridae* [67]. Two types of OBs were reported by Ramasamy et al. [81]: Type 1 with paracrystalline array of polyhedrin sub-units containing numerous occluded and a few non-occluded virions enclosed by a double envelope; Type 2 consisted of non-crystalline, granulin-like sub-units containing mostly non-occluded virions. Mari et al. [67] also reported three polyhedrin polypeptides: a 56 kDa major polypeptide and two minor polypeptides (47 and 49 kDa, respectively). However, Chang et al. [15] had reported a 62 kDa polyhedrin from *Penaeus monodon*-type baculovirus. According to one hypothesis, these minor differences in the molecular weight would reflect the diversity of this baculovirus, as well as the existence of many MBV-type diseases and BP-type diseases which could share similar gross characters like OB shape, but differ by some minor characters such as size of OBs and molecular weight of polyhedrin [67]. Although nudiviruses have been thought to be non-occluded viruses, PmNV/MBV is occluded. However, the gene encoding OB in MBV does not show any similarity with any of the sequences available in public database and has no homologues to the OB genes of baculoviruses [13]. Nevertheless, the gene sequence of other nudiviruses showed homologue of lepidopteran- and hymenopteran-type polyhedrin/granulin gene, though it is not known whether these genes functionally encode for OB matrix protein [106].

Studies conducted by Vickers et al. [103] on MBV from Australia reported 1–3 OBs in infected nuclei and large number of unoccluded virions in hypertrophied nuclei. OBs showed mature virions but not as densely packed as in the nucleoplasm. They also reported the distribution of virions in groups throughout the nucleus rather than close to nuclear membrane or nucleolus. This is in contrast to the observation made by Johnson and Lightner [44]. Vickers et al. [103] proposed a model for MBV viral life cycle based on the ultrastructural observation of electron dense, naked nucleocapsids, which are presumably carrying viral

Table 2 Comparative features of three strains of SNPV reported from penaeid shrimp

Strain	Host	Geographic location	Nucleocapsids (nm)	Enveloped virions (nm)	References
MBV	<i>Penaeus monodon</i>	Indo-pacific	$246 (\pm 15) \times 42 (\pm 3)$	$324 (\pm 33) \times 75 (\pm 4)$	[10, 51, 56]
		India	$161 (\pm 3) \times 54 (\pm 2)$ (Type 1) $295 (\pm 1) \times 67 (\pm 1)$ (Type 2)	$267 (\pm 2) \times 78 (\pm 3)$ (Type 1) $326 (\pm 4) \times 73 (\pm 1)$ (Type 2)	[81]
		Taiwan	$250\text{--}269 \times 62\text{--}68$	$265\text{--}282 \times 68\text{--}77$	[67]
	<i>Macrobrachium rosenbergii</i>	Thailand	–	$327 \pm 29 \text{ nm} \times 87 \pm 12 \text{ nm}$	[40]
PBV	<i>Penaeus plebejus</i> , <i>P. monodon</i> , <i>P. merguensis</i>	Australia	$260\text{--}330 \times 45\text{--}52$	420×60	[10, 51]
BBV	<i>Metapenaeus bennettiae</i>	Australia	$238\text{--}288 \times 37\text{--}38$	$300\text{--}387 \times 54\text{--}56$	[51, 84]

genome, found free in the cytoplasm and attached to the nuclear membrane. They also observed empty nucleocapsids at the nuclear membrane and suggested that the envelope is lost at the plasma membrane and the nucleocapsid traverses the cytoplasm and attaches to the nuclear membrane through which the viral genome enters the nucleus. Similar observation of naked, empty capsid aligned at the nuclear pore was reported by Johnson and Lightner [44] for MBV and Couch [27] for Baculovirus penaei. Further, in the case of MBV, Johnson and Lightner [44] and Vickers et al. [103] noticed strand-like material, presumably nucleic acid, streaming through the nuclear pore. Flegel et al. [36], while studying MBV from Thailand also noticed similar nucleic acid filament. These observations suggest that MBV has similar mechanism of invasion of cells as that of nuclear polyhedrosis and granulosis viruses of insects. Based up on all these observations, Vickers et al. [103] presented a model for MBV virogenesis and replication.

Genome

Electron microscopic study of extracted MBV nucleic acid reveals a circular, double-stranded DNA with a genome size in the range of 80–160 kbp [67] and the size is reported to be similar to that of the dsDNA of insect baculoviruses. Further, recent information on the genome sequence of other nudiviruses showed that the genome size of HzNV-1, *Oryctes rhinoceros* nudivirus (OrNV) and *Gryllus bimaculatus* nudivirus (GbNV) are 228,089, 127,615 and 96,944 bp, respectively with 98–154 ORFs [22, 108, 109]. However, for MBV, only partial sequences are available in the GenBank and the full genome sequencing is in progress. However, alignment of available partial sequences of Philippine isolate and five sequences of Indian and one sequence of Taiwan isolates of MBV revealed 87 % homology among these isolates [12]. Further, phylogenetic tree showed distinct clades with Philippine and Indian isolates showing separate clusters. However, one of the Indian isolates formed a distinct clade and another one clustered with the Taiwanese isolate. The authors also indicated that the interesting clustering pattern of Indian MBV could be due to the fact that apart from the presence of distinct Indian strains of MBV, the virus from Taiwan could have been introduced through importation of shrimp into India. Further, according to these authors, the presence of an intermediate clade could be the result of small mutation in the genome due to the interaction of different strains. This observation is very significant in the context of developing a consensus PCR-based detection system for MBV in India.

Diagnosis

MBV infection is one of the easiest to diagnose by direct microscopic examination. Live larvae and postlarvae can be directly observed under a light microscope through the transparent carapace for the presence of OB in the hepatopancreas which is characteristic of MBV infection. The OB can be easily seen as small, raised, spherical clusters of polyhedral particles which can be easily differentiated from the lipid droplets with experience. The OB are more evident under a phase interference microscopy [31] measuring 0.1–20 μm . Alternatively, the OB can be observed in a squash mount preparation of freshly collected hepatopancreas samples stained with 0.05 % malachite green [49]. The OB stained green appear as clusters in the enlarged nucleus (Fig. 1). This can also be done by staining squash preparation of hepatopancreas by hematoxylin–eosin (H&E), and the occlusions appear eosinophilic (Fig. 2). The OB can also be seen in the midgut epithelial cells and fresh fecal strands [51]. Fecal strands offer the added advantage that it provides non-lethal sampling for valuable broodstock.

The OB can be observed under light microscope in stained sections of hepatopancreas and anterior midgut. The OB are seen in the hypertrophied nucleus and the tubules of hepatopancreas with fragmented and marginated chromatin (Figs. 3, 4). The OB are usually restricted to the hepatopancreas and the mid gut epithelium. The sensitivity of the detection of MBV can be increased by either using epifluorescence microscopy and acridine orange staining [23, 29] or staining the squash mount of hepatopancreas and faecal samples and tissue sections with aqueous phloxine

Fig. 1 Squash preparation of hepatopancreas of *Penaeus monodon* infected with monodon baculovirus stained with 0.05 % aqueous malachite green. Arrows indicate the characteristic multiple inclusions of MBV

Fig. 2 Tissue impression smear of hepatopancreas of *Penaeus monodon* infected with monodon baculovirus stained with hematoxylin and eosin. Arrow indicates the characteristic eosinophilic multiple inclusions of MBV

Fig. 3 Histological section of hepatopancreas of *Penaeus monodon* infected with monodon baculovirus stained with hematoxylin and eosin. Arrows indicate the characteristic eosinophilic multiple inclusions of MBV

and examining under fluorescent microscope [31, 93]. The OB appear bright yellow green against a pale green background [51]. The infection can also be diagnosed by in situ hybridization in fixed tissue sections [51, 55, 79] or by dot blot hybridization with purified DNA [16] using DIG-labeled probes. Dot blot hybridization using hepatopancreas or fecal homogenate is not recommended as it gives false positive and false negative results [51]. Electron microscopic observation of rod-shaped virus of $\sim 360 \times 70$ nm embedded in the OB can be used for confirmatory diagnosis [31]. Electron microscopic studies on MBV have been reported by many workers [44, 52, 53, 56].

Fig. 4 Enlarged view of the histological section of hepatopancreas of *Penaeus monodon* infected with monodon baculovirus stained with hematoxylin and eosin. Arrows indicate the hypertrophied nuclei with margined chromatin and inclusions of MBV

Fig. 5 PCR amplification of MBV from *Penaeus monodon* hepatopancreas DNA. Lanes 1–2: clinical samples, M = 1 kb Plus DNA ladder

ELISA can be used to detect MBV in tissue homogenates using polyclonal antibodies developed against the OB [43]. Immunological diagnosis using polyclonal antibodies or monoclonal antibodies raised against MBV polyhedrin has also been reported [8, 83, 86].

Polymerase chain reaction (PCR) has been extensively used in the sensitive detection of MBV [7, 16, 61, 62, 102] (Fig. 5). PCR for polyhedrin gene with primers designed using the conserved regions of insect polyhedrin gene has been used to detect the infection. However, the primers reported also amplified AcMNPV, an insect baculovirus. Hsu et al. [43] designed primers to amplify DNA polymerase gene of MBV which specifically detected MBV and not other baculoviruses. Molecular diagnosis for improved

detection based on one-step PCR amplifying 261 bp MBV-specific sequences has been reported [89]. Similarly, PCR assay to detect specific strain such as Philippine MBV has also been developed [11]. Dual infection of MBV and WSSV being reported in many countries [28, 39, 42, 76] a sensitive duplex PCR has been developed to detect both the viruses [73]. A TaqMan probe based [112] real-time PCR have been developed to quantify the viral load. Further, a loop mediated isothermal amplification (LAMP) has been reported by Chaivisuthangkura et al. [14]. A recent addition to the array of diagnostic tools to detect MBV is the LAMP combined with a lateral flow dipstick [74].

Taxonomy and Phylogenetic Position

Taxonomic position of MBV is still uncertain and the International Committee on Taxonomy of Viruses (ICTV) lists MBV (spherical baculovirus) as a tentative species named *PemoNPV* in the genus *Nucleopolyhedrovirus* [91]. The confusion in the taxonomy of the virus is due to the fact that the relationship of MBV to other occluded and non-occluded bacilliform viruses is not clear, and the whole genome sequence of the virus is also not available [40]. Based on the genomic comparison of intracellular bacilliform insect viruses that do not produce polyhedron, it has been suggested to create a new group of non-occluded bacilliform viruses called nudiviruses [108]. Subsequently, it has also been revealed that similarities do exist between a hypothetical gene sequence of MBV and the sequence of non-occluded lepidopteran virus *Heliothis zea* NV-1 (Hz1) [106]. Based on this, these authors have proposed that formation of OB was a result of parallel evolution and should not be used as a criterion for designating family or sub-family. However, there are strong indications that nudiviruses and baculoviruses are closely related to each other than to other known viruses based on the conserved nature of 20 baculovirus core genes in nudiviruses [106]. According to Wang and Jehle [106], nudiviruses comprise five tentative species which include four insect viruses and *PmNV*/MBV, and based on their distinct biological and ecological features and virion properties they have suggested that an independent family '*Nudiviridae*' within a new order '*Baculovirales*' along with the *Baculoviridae* may be established to accommodate all nudiviruses.

Concluding Remarks

Although MBV is not a serious pathogen, it can cause severe damage when the infected animals are stressed due to poor water quality and by predisposing the animals to

secondary bacterial infections and other viruses. Hence the virus has to be eradicated from cultured stocks. Since the virus is embedded in OB and is more resistant to disinfectants than other shrimp viruses, appropriate disinfectants at correct dosage have to be used to disinfect farms, hatcheries and fomites. Along with disinfection, eradication of infected broodstock, stocking of disease-free postlarvae and maintaining optimum water quality parameters need to be practiced to reduce the loss due to MBV. Complete genome sequence information, studies on strain variations and effective disinfection protocols can help in better understanding of the host-pathogen interaction and effective management of the disease.

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