Chapter 9
Nanotechnologies in the Health Management of Aquatic Animal Diseases

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9.1 Introduction

Aquaculture, also known as "underwater agriculture," is the fastest-growing food-producing sector in the world. With the growth of aquaculture, consumers from low- to high-income nations have benefited from year-round availability and access to aquatic foods, which are rich in protein and micronutrients (Belton and Thilsted 2014; Béné et al. 2016; Thilsted et al. 2016; Belton et al. 2020). Among the continents, Asia contributes more than 90% of the world's aquaculture production (Bondad-Reantaso et al. 2005). Aquaculture encompasses a very wide range of different aquatic farming practices, including those involving crustaceans, fish, seaweeds, molluscs, and other aquatic species. More than 14.5 million people are dependent on aquatic farming practices for their livelihoods, food security, especially in rural communities, and poverty alleviation such as income generation and employment (Kumar et al. 2015).

With the rapid development of aquaculture in India, disease outbreaks remain the biggest challenge to the sustainability of aquaculture production. Viral and bacterial pathogens are a chronic risk for the aquaculture sector and its highly intensive fish farming practices (Kennedy et al. 2016). On the other side, increased trade and

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V. K. Arivarasan et al. (eds.), *Nanotechnological Approaches to the Advancement of Innovations in Aquaculture*, Nanotechnology in the Life Sciences, https://doi.org/10.1007/978-3-031-15519-2_9

supply of seeds including fish fry and shrimp larvae are often of low quality and affected by diseases (Stentiford et al. 2012). Many aquaculture systems, however, still lack the motivation to meet sustainability criteria because their targeted markets do not reward producers, leading to poor management and causing severe mortalities and economic losses to the industry (Walker and Winton 2010). Outbreaks prompted by diseases increase the use of antibiotics and other chemicals, increasing environmental risks and the lack of adequate biosecurity control.

In India, several transboundary aquatic animal diseases have caused massive economic losses, which include the spread and outbreaks of new emerging viral diseases like cyprinid herpesvirus 2 (CyHV-2), carp edema virus (CEV), viral nervous necrosis (VNN), red sea bream iridovirus disease (RSIVD), infectious spleen and kidney necrosis virus (ISKNV), and tilapia lake virus (TiLV). A few viral diseases have also been reported in shrimps: monodon baculovirus (MBV), white spot syndrome virus (WSSV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), hepatopancreatic parvo-like virus (HPV), white tail disease (WTD), and infectious myonecrosis virus (IMNV).

The term nanotechnology refers to a multidisciplinary field which can be applied to various aspects of applications, which include large-scale industries, medicine, agriculture, etc. The discovery of nanoparticles in this modern world has brought fields of research and applications towards each other, leading to an amalgam of sciences. The nanoparticles are the particles which are formed in the size range of 1–100 nm, and this changes the physical and chemical properties of their bulk counterparts (Abou El-Nour et al. 2010). In the application of nanotechnology to aquaculture, the role of nanotechnology diversifies into many forms. The effects of nanotechnology and nanoparticles on aquaculture can be found in drug delivery development, food packaging, and many more (Abou El-Nour et al. 2010; Sannino 2021).

9.2 Nanotechnology in Aquaculture

Nanomaterials and their technology can be utilized for many applications, including diagnostics and drug delivery. Some of the reports have been elaborated here; Saleh and El-Matbouli (2015) reported the gold nanoparticles to be used to develop and evaluate a specific and sensitive hybridization assay for direct and rapid detection of the highly infectious pathogen termed cyprinid herpesvirus 3. A duplex PCR was developed for the detection of the cyprinid herpesvirus 2 (Luo et al. 2014), a droplet digital PCR for the detection of the cyprinid herpesvirus 2 (Hao et al. 2016), and similarly, the loop-mediated isothermal amplification assay for the rapid detection of the cyprinid herpesvirus 2 in Gibel carp was also developed (Zhang et al. 2014).

There are reports of nanoparticles being utilized as drug delivery agents, which have proven to be very much successful. The DNA vaccines against sea bass nodavirus-based chitosan nanoparticles have shown good results (Vimal et al. 2014), and some suggested that PLGA-based nanoparticles could be used for drug

delivery (Adomako et al. 2012; Embregts and Forlenza 2016; Kole et al. 2019b). The delivery systems and their framework improve solubility and bioavailability and also lead to sustained release of hydrophobic drugs, thereby protecting the chemical payload from degradation and facilitating the cellular and tissue targeting (Hu et al. 2018; Oroojalian et al. 2020; Venkatesan et al. 2013). The utilization of chitosan and polycaprolactone polymers for the delivery of ascorbic acid was reported (Luis et al. 2021). Many researchers have reported on the utilization of the vaccine delivery for fish (Abbas 2021; Adams 2019; Bedekar and Kole 2022; Heyerdahl et al. 2018; Huang et al. 2021; Jeong et al. 2020; Kayansamruaj et al. 2020; Somamoto and Nakanishi 2020; Thirumalaikumar et al. 2021; Wang et al. 2020; Zhang et al. 2020; Zhang et al. 2021; Zhao et al. 2020; Zhu et al. 2020). There are also reports on the nano-based delivery of drugs and chemicals for aquaculture applications (Ji et al. 2015; Kole et al. 2019a; Li et al. 2013; Thirumalaikumar et al. 2021; Thwaite et al. 2018; Zhang et al. 2020; Zhang et al. 2021; Zhu et al. 2020). There is growing interest in developing a more sustainable and targeted delivery system for drugs and vaccines for aquatic animals. The role of the nanoparticles can be utilized for the definite release of the drug payload in a sustained manner.

9.3 Fish Viral Diseases

9.3.1 Cyprinid Herpesvirus 2 (CyHV-2)

Cyprinid herpesvirus 2 (CyHV-2) is an emerging pathogen of the alloherpesviridae and is also known as herpesviral haematopoietic necrosis virus (HVHNV) of goldfish or goldfish haematopoietic necrosis virus (GFHNV) (Hanson et al. 2011). CyHV-2 infection has been reported globally, including in Asia (Waltzek et al. 2009), Europe (Boitard et al. 2016; Doszpoly et al. 2011; Jeffery et al. 2007), North America (Goodwin et al. 2006), and Oceania (Becker et al. 2014). At present, CyHV-2 has been reported in 16 countries, including India (Sahoo et al. 2016). CyHV-2 belongs to the family Alloherpesviridae and the genus Cyprinivirus. CyHV-2 has an icosahedral capsid with a linear dsDNA of about 290 kbp and encodes approximately 150 genes (Davison et al. 2013). Host-range studies for CyHV-2 have been limited; previously, CyHV2 was considered a pathogen of goldfish. To date, it has been able to infect a much wider range of cyprinid species, including crucian carp (*Carassius carassius*) and Prussian carp (*C. gibelio*) (Xu et al. 2013; Fichi et al. 2016; Zhao et al. 2019).

CyHV-2 causes acute disease in all ages of goldfish, and mortality occurs while infection can reach almost 100% at temperatures between 15°C and 25°C (Goodwin et al. 2009; Davison et al. 2013). The entry of the virus is unknown; an infected fish can transmit the virus horizontally, and vertical transmission is also possible from parent fish to offspring (Goodwin et al. 2009). Diseased fish show no remarkable external signs except pale gills, which result from severe destruction of the

hematopoietic tissues (Jung and Miyazaki 1995). The most severe gross changes in the infected fish are swollen kidneys, an empty intestine, and splenomegaly with white nodular lesions in the spleen (Jung and Miyazaki 1995; Jeffery et al. 2007). According to histological identification, infected cells may have enlarged nuclei with margination of chromatin and the presence of intranuclear inclusion bodies (Jeffery et al. 2007). Viral DNA may be detected for longer periods in healthy goldfish brood stock due to the latency of CyHV-2 (Goodwin et al. 2009). There is no commercial vaccine or effective treatment available for CyHV-2 (Thangaraj et al. 2021). The virus remains a serious threat to the Indian ornamental fish trade and food fish aquaculture. To reduce and control the spread of the virus, it is necessary to follow strict biosecurity measures and regulate fish trading.

9.3.2 Carp Edema Virus (CEV)

Carp edema virus (CEV), which causes carp edema virus disease (CEVD) or koi sleepy disease (KSD), is an emerging disease of global concern. CEV is considered a potential risk for the koi trade and for global carp aquaculture, because of its wide distribution and potential virulence. CEV was first detected in koi carp in Japan in 1974 (Murakami 1976). Due to the global trade in live ornamental fish, the virus has spread to other countries such as the USA, Czech Republic, Austria, India, Germany, the Netherlands, Italy, China, Brazil, Poland, Hungary, and South Korea (Rehman et al. 2020). Koi carp culture in India is growing rapidly in many states, including Kerala, Tamil Nadu, West Bengal, and Maharashtra. Unfortunately, the outbreak of CEV has occurred in koi farms in Kerala, India (Swaminathan et al. 2016). Koi carp and common carp are known to be species susceptible to CEV, with high mortality rates during the outbreaks reaching up to 75–100% (Miyazaki et al. 2005; Way and Stone 2013).

Currently, limited information is known about the virus that causes KSD. Clinical signs of KSD are typical sleepy behaviour, enophthalmia, a generalized oedematous condition, and gill necrosis (Lewisch et al. 2015). CEV is an unclassified double-stranded DNA virus and belongs to family Poxviridae. The size of the immature and mature virion is about 416–450 nm and 300–400 × 250–400 nm in diameter, respectively (Adamek et al. 2017).

The clinical signs of KSD and KHVD are very similar, and the disease can be misidentified easily. KSD diagnosis relies on PCR, and real-time PCR assays have been developed and validated for the detection of CEV infection (Oyamatsu et al. 1997; Matras et al. 2019). Attempts have also been made to replicate the CEV *in vitro*, but employing the fish cell lines presently available has not been successful (Jung-Schroers et al. 2016; Lewisch et al. 2015; Swaminathan et al. 2016). Although CEV has been present in aquaculture for many years, there is still a lack of knowledge about its transmission among species, which is important to prevent the spread of CEV/KSD (Matras et al. 2019). Until now, there have been no treatments or prophylactic measures available for KSD. Extreme precautionary measures and

regulations are necessary in the live fish trade in order to prevent the further spread of CEV infection (Swaminathan et al. 2016).

9.3.3 Viral Encephalopathy and Retinopathy (VER)

Viral encephalopathy and retinopathy or viral nervous necrosis is an OIE significant disease. Viral nervous necrosis (VNN) infects fishes with so-called piscine nodaviruses and belongs to the family Nodaviridae. First isolated Nodaviridae is the striped jack nervous necrosis virus (SJNNV) and genus *Betanodavirus* (Mori et al. 1992). The virus has been recorded in over 120 different fish species, and the mortality associated with the disease is severe, reaching 100% depending on age, with younger fish being more susceptible (Munday et al. 2002; Costa and Thompson 2016). The disease, designated viral nervous necrosis (VNN) when it was first described in 1990 (Yoshikoshi and Inoue 1990), is also known as viral encephalopathy and retinopathy (OIE 2003). Subsequently, the disease has been reported globally, including in India in 2005 (Azad et al. 2005).

The VNN is classified under the family Nodaviridae and the genus *Betanodavirus*. The virus consists of a larger genomic segment of single-stranded positive-sense RNA (composed of two segments), which encodes the RNA-dependent RNA polymerase. The coat protein is encoded by RNA 2 (1.4 kbp), and RNA 3 of 0.4 kb encodes the protein B2. It has non-enveloped isometric symmetry (icosahedral) and is about 30 nm in diameter. Till now, nodaviruses have been classified by different clades: the striped jack clade (SJNNV), the red-spotted *grouper* clade (RGNNV), the tiger puffer clade (TPNNV), the barfin flounder clade (BFNNV), *Dicentrarchus labrax* encephalitis virus (DLEV), the Japanese flounder nervous necrosis virus (JFNNV), *Lates calcarifer* encephalitis virus (LcEV), Atlantic halibut nodavirus (AHNV), and the Malabar grouper nervous necrosis virus (MGNNV). Based on the optimal growth temperatures, some genotypes have been classified, e.g., 25–30°C for the RGNNV genotype, 25–30°C for the SJNNV genotype, 20°C for the TPNNV genotype, and 15°C for the BFNNV genotype, and differ among the other genotypic variants.

Investigations on the possible routes of VNN infection by horizontal and vertical transmission are also possible. Horizontal transmission is the common mode of infection for betanodavirus, and it has been demonstrated in European sea bass (Peducasse et al. 1999; Skliris and Richards 1999) and by the cohabitation of sea bream (*Sparus aurata*), an asymptomatic carrier of fish nodavirus in sea bass (Castric et al. 2001). Vertical transmission of the virus has been identified in various fish species (Arimoto et al. 1992; Grotmol et al. 1999; Grotmol and Totland 2000; Breuil et al. 2002). The virus localizes in the brain, spinal cord, and retina of affected fish and is characterized by neurological abnormalities (erratic swimming, spiral movements with belly-up) and a distinct vacuolization of the nerve tissue (brain and retina) (OIE 2006). The distribution of VNN in surviving fish was not only in the CNS but also in other tissues like gonad, intestine, stomach, kidney and liver of

brood stock carriers (Arimoto et al. 1992; Munday et al. 2002). Moreover, the virus could spread between farms through contaminated personnel and farm equipment; hence, adequate biosecurity measures should be ensured to prevent its spread. Recently, scientists (ICAR-CIBA) found the remedy for this disease in India, and they developed a recombinant vaccine for VNN affecting several fish species under the commercial name Nodavac-R.

9.3.4 Red Sea Bream Iridovirus (RSIV)

Red sea bream iridovirus (RSIV), in the genus *Megalocytivirus* (Kurita and Nakajima 2012), is a virus causing extensive economic loss to the aquaculture industry. RSIVD has caused severe mortality in many cultured and wild fish species of several countries (OIE 2019) including India. The first outbreak of this pathogen was reported in farmed Asian sea bass (*Lates calcarifer*) in 2018 (Girisha et al. 2020). RSIV infects more than 30 other species of cultured marine fish (Matsuoka et al. 1996; Kawakami and Nakajima 2002).

RSIV belongs to the family Iridoviridae and the genus Megalocytivirus. It has identified three genotypes: red sea bream iridovirus (RSIV), infectious spleen and kidney necrosis virus (ISKNV), and *turbot reddish* body iridovirus (TRBIV) (Subramaniam et al. 2012). RSIV is an enveloped virus with icosahedral virions that are found within the cytoplasm of enlarged cells, and the virion size is about 200–240 nm in diameter. It is a double-stranded DNA virus with a genome size of 111.15 KB (Shiu et al. 2018; Puneeth et al. 2021). RSIV may spread not only in the summer, when this disease is prevalent, but also in the winter. The horizontal mode of transmission is the route for causing RSIV infection, and vertical transmission of this virus infection is not known. Clinical signs of the infected fish include lethargy, helpless swimming, severe anemia, petechiae of the gills, and enlargement of the spleen, with 20–60% mortality. Histopathology is characterized by the development of enlarged cells in the spleen, heart, kidney, liver, and gills and the observation of basophilic characteristics in Giemsa staining.

An effective formalin-inactivated vaccine was developed by Nakajima et al. (1999). Currently, for this virus, the injectable vaccine is now commercially available in Japan for red sea bream, striped jack (*Pseudocaranx dentex*), Malabar grouper (*Epinephelus malabaricus*), and orange-spotted grouper (*Epinephelus coioides*). In a recent report from India, RSIV infection has been found in farmed fish (Girisha et al. 2020). There is a potential threat from this virus that can hamper the industry. Hence, active research must be carried out on RSIV for its prevention and spread in India.

9.3.5 *Infectious Spleen and Kidney Necrosis Virus (ISKNV)*

Infectious spleen and kidney necrosis virus (ISKNV) is an emerging and transboundary fish pathogen that causes large-scale mortalities in fish. ISKNV belongs to the family Iridoviridae and the genus Megalocytivirus and is closely related to RSIV. Among these, ISKNV causes both symptomatic and asymptomatic infections in about 50 different freshwater, brackish, and marine species, particularly during the summer (Kurita and Nakajima 2012).

Since the first report of ISKNV in Chinese mandarin fish, Siniperca chuatsi (He et al. 2002), ISKNV has been reported in Australia (Go and Whittington 2006), Korea (Song et al. 2008), Singapore (Jeong et al. 2008), Malaysia (Subramaniam et al. 2012), Germany (Jung-Schroers et al. 2016), and Indonesia (Sukenda et al. 2020). Recently, ISKNV was reported in asymptomatic exotic ornamental fishes in India (Girisha et al. 2021). Global live ornamental fish trade without the compliance of appropriate pathogen screening and quarantine serves as the major reason for the transboundary spread of ISKNV (Jung-Schroers et al. 2016; Rimmer et al. 2015).

The genome size of ISKNV is 111 kb, and it has about 124 open reading frames (ORFs) (He et al. 2001). The virus is icosahedral in shape, encapsulated with a nucleocapsid structure, and the size of the virus ranges between 110 and 150 nm (Shi et al. 2004; Jung-Schroers et al. 2016). Clinical signs of ISKNV-infected fish show symptoms such as being lethargic and exhibiting severe anemia, petechiae of the gills, enlargement of the spleen, abnormal swimming, ulceration, haemorrhages, and a darkened body. ISKNV can cause severe infection at various stages of the life cycle, and moreover, it can cause up to a 75% mortality rate. In particular, in India, ISKNV is currently considered an exotic pathogen. However, considering the ornamental trade, it is very important to have a monitoring and surveillance program to screen for emerging and re-emerging diseases and develop preventive and control measures.

9.4 Tilapia Lake Virus Disease

Recently, tilapia lake virus (TiLV) has been noted as an important infectious agent that may pose a serious threat to the global tilapia industry (Jansen et al. 2019; Mugimba et al. 2018; Pulido et al. 2019). TiLV has been taxonomically classified as a Tilapia tilapinevirus species, in the *Tilapinevirus* genus, Amnoonviridae family (ICTV 2018). To date, TiLV has been reported in 16 tilapia-farming countries, including Asia, Africa, the Middle East, and South and Central America (Taengphu et al. 2020). The disease is caused by a novel orthomyxo-like virus also known as TiLV (Bacharach et al. 2016), and mortality rates ranging from 80% to 90%, especially in fingerlings and juveniles, have been reported following infection with TiLV (Fathi et al. 2017; Surachetpong et al. 2017). The clinical signs of the disease are lethargy, haemorrhages, abdominal swelling, exophthalmia, and severe mortalities

in farmed and wild populations of tilapia, which are characterized by syncytia formation in the liver of affected fish. The condition is known as syncytial hepatitis of tilapia (Ferguson et al. 2014).

TiLV virions are enveloped in a round or oval shape, 55–100 nm in diameter, and a single-stranded RNA virus which contains 10 segments encoding 10 proteins. The total viral genome size is about 10,323 kb (Eyngor et al. 2014; Bacharach et al. 2016; Del-Pozo et al. 2017). The genome organization and ultrastructural morphology of TiLV resemble those of other orthomyxoviruses (Del-Pozo et al. 2017; Eyngor et al. 2014). A total of 20 complete TiLV genome sequences from six different countries are available, including 10 from Thailand, 3 from Bangladesh, 2 each from the USA and Israel, and one each from Peru, Ecuador, and India (Verma et al. 2022). In experimental trials, TiLV infection has been reported in tilapia to be associated with 70–90% mortality by intra-peritoneal (i.p.) injection (Mugimba et al. 2019) and cohabitation (Liamnimitr et al. 2018). To date, TiLV affects several species of cultured tilapia cichlids, including Nile tilapia (Tattiyapong et al. 2017), red tilapia (Tattiyapong et al. 2017; Mugimba et al. 2019), Mozambique tilapia (Nanthini et al. 2019) hybrid tilapia (Mugimba et al. 2019; Amal et al. 2018), and several wild tilapia (Eyngor et al. 2014). A recent experimental study has reported that other than tilapia species, giant gourami (Jaemwimol et al. 2018) and wild tinfoil barb (Abdullah et al. 2018) have also been found to be susceptible to TiLV infection.

TiLV can be transmitted both horizontally and vertically. Horizontal transmission is considered to be the major route for the spread of TiLV, with the virus being transmitted to healthy fish through direct contact with the skin and mucus of infected fish or through cannibalism of moribund or dead fish (Liamnimitr et al. 2018; Tang et al. 2021). Vertical transmission of TiLV has also been observed in natural cases of infection with TiLV and following experimental infection (Dong et al. 2020).

However, TiLV has been reported as a cause of mass mortality in tilapia in India (Behera et al. 2018). Since it is a viral disease, therefore, there is no effective treatment for it. In response to this, active surveillance for the presence or absence of the virus/disease was carried out. Some precautionary measures include banning the importation of tilapia from TiLV-confirmed countries. Other effective measures for control of the disease include a combination of biosecurity measures, the breeding of fish with improved resistance, and the development of new vaccination against the TiLV infection disease.

9.5 Shrimp Viral Diseases

9.5.1 Monodon Baculovirus (MBV)

Monodon baculovirus (MBV), the causative agent of spherical baculovirosis, was the first virus reported in penaeid shrimp, described by Lightner and Redman (1981) from *Penaeus monodon* in Taiwan. MBV should be reclassified as *P. monodon*

nudivirus (PmNV) and reassigned to the Nudiviridae (Wang and Jehle 2009; Yang et al. 2014). MBV infects *P. monodon*, *P. merguiensis*, *P. semisulcatus*, *P. kerathurus*, *P. vannamei*, *P. esculentus*, *P. penicillatus*, *P. plebejus*, and *M. ensis.* MBV was found to infect up to 20% of populations of wild *P. monodon* in Asia (Manivannan et al. 2004; Leobert et al. 2008). MBV has been detected in *P. monodon* in Taiwan, the Philippines, Malaysia, French Polynesia, Hawaii, Kenya, Mexico, Singapore, Indonesia, Israel, and Thailand. In India, the prevalence of MBV and mortalities in hatcheries and farms associated with MBV have been reported (Ramasamy et al. 1995; Karunasagar and Karunasagar 1998). Artemia does not act as the mechanical carrier for MBV (Sarathi et al. 2008). Vijayan et al. (1995) reported the presence of MBV in *P. indicus* along the southeast coast of India. MBV can infect *Macrobrachium rosenbergii* and produce lesions in the hepatopancreas similar to those in *P. monodon* at the early stage of infection (Gangnonngiw et al. 2010). There are about nine distinctly different isolates obtained from shrimp samples, which confirmed the existence of variation in the strains of MBV in India reported by Suganthi et al. (2012).

Symptoms of the disease include a reduction in feeding and growth, reduction in activity, and dark outgrowth on the gill surface of the shrimp. Mortalities occur primarily among post-larvae in the hatchery, although disease may also occur in juvenile and adult prawns (Johnson and Lightner 1988). Adult brood stock can carry the virus and transmit MBV to offspring via horizontal transmission (Paynter et al. 1992), direct from the water column, or cannibalism, and it is believed, but not proven, that transmission can also be vertical from brood stock to offspring. Infection can result in substantial economic loss due to poor performance in growth and reduced survival of post-larvae—up to 90% at high densities. Furthermore, stress and overcrowding are the predisposing factors that may increase the severity of MBV infection (Lightner et al. 1983a).

The MBV particles are rod-shaped and replicate in the nucleus. These appear either free or within proteinaceous polyhedral occlusion bodies and contain DNA. The nucleocapsids of MBV measure 42±3 nm by 246±15 nm, while the enveloped virions are larger, measuring 75±4 nm by 324± 33 nm (Lightner et al. 1983a; Chen et al. 1989; Brock and Lightner 1990). However, little is known about the mechanism of MBV production in the host cell. Diagnosis of MBV depends upon the demonstration of MBV occlusion bodies in hypertrophied nuclei of anterior midgut epithelium and hepatopancreatic cells by direct light microscopy or standard H&E staining (Lightner and Redman 1998). However, the detection of occlusion bodies requires a high level of infection, thus these traditional methods have limited sensitivity. Applications of molecular techniques, including in situ hybridization (Poulos et al. 1994) and polymerase chain reaction (Belcher and Young 1998), have been developed as more sensitive methods for detecting MBV.

9.6 White Spot Syndrome Virus (WSSV)

The WSSV has been one of the major threats to the shrimp industry over the past two decades, which is responsible for huge economic loss in the shrimp culture industry not only in India but also worldwide. The global loss to the shrimp culture industry due to this virus has been estimated to be about US$ 10 billion (Stentiford et al. 2009). The loss in India alone has been estimated to be about several million dollars per year, and the loss continues to threaten the long-term sustenance of the shrimp industry in India. WSSV was first reported in 1992 in Taiwan, from where it spread to all shrimp-growing countries (Flegel 1997). Disease outbreaks can reach a cumulative mortality of up to 100% within 3–7 days of infection (Escobedo-Bonilla et al. 2008). This is a very fast-reproducing, widely spreading, and highly virulent crustacean pathogen. WSSV has a large host range, infecting shrimp, crayfish, and lobster, among many other species (Sánchez-Paz 2010). A total of 98 potential host species for WSSV have been identified from the scientific literature (Stentiford et al. 2009).

The virus belongs to the family Nimaviridae, the genus *Whispovirus*, and contains a double-stranded DNA genome with sizes ranging from 292.9 to 307.2 kb (Sánchez-Martínez et al. 2007). The intact virus is enveloped and elliptical in shape, measuring 266 nm. The nucleocapsid of WSSV is cylindrical in shape (420 nm), with one end flat and the other pointed, and has a pattern of opaque and transparent striations arranged perpendicularly to the long axis of the nucleocapsid (Sahul Hameed et al. 1998). The viral DNA encodes major structural proteins and minor proteins for various functions, including pathogenesis (Van Hulten et al. 2001a; Van Hulten et al. 2001b; Van Hulten et al. 2002).

Clinical signs of WSSV include a sudden reduction in food consumption, lethargy, loose cuticle, often reddish discolouration, and the presence of white spots on the inner surface of the carapace and cuticle over the abdominal segments (Takahashi et al. 1994). Transmission of the virus occurs mainly through oral ingestion and waterborne routes in farms and by vertical transmission in the case of shrimp hatcheries (Rosenberry 2003). White spot syndrome virus spreads through cannibalism of sick or dying shrimp or through contaminated water. Birds can carry infected shrimp between ponds. The virus can survive and remain infective in seawater for 4–7 days without a host.

To date, measures to control WSSV have included improving environmental rearing conditions and management practices (Rahman et al. 2007), using specialized formulated diets to boost shrimps' immune systems (Rajkumar et al. 2017), as well as using vaccines (Rijiravanich et al. 2008).

9.6.1 *Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV)*

Infectious hypodermal and hematopoietic necrosis virus (IHHNV) is a widely distributed single-stranded DNA parvovirus that has been responsible for major losses in wild and farmed penaeid shrimp populations along the northwestern Pacific coast of Mexico since the early 1990s (Robles-Sikisaka et al. 2010). IHHNV was first described as the cause of acute epizootics and mass mortalities (> 90%) in juvenile and sub-adult *L. stylirostris* farmed in super-intensive raceway systems in Hawaii (Brock 1983; Lightner et al. 1983b, 1983c). Natural infections have been reported from *P. stylirostris*, *P. vannamei*, *P. occidentalis*, *P. californiensis*, *P. monodon*, *P. semisulcatus*, and *P. japonicus. P. setiferus*, *P. dourarum*, and *P. aztecus* have been infected experimentally with IHHNV, and *P. indicus and P. merguiensis* appear to be refractory to infection (Brock and Lightner 1990; Lightner 1996a).

The clinical signs of IHHNV disease in *P. stylirostris* are nonspecific and include anorexia, lethargy, and erratic swimming. Infected shrimps have been observed to rise to the surface of the water, remain motionless for a few moments, then roll over and sink to the bottom. Mortality may exceed 90% within several weeks of the onset of infection in juvenile *P. stylirostris* (Bell and Lightner, 1987). The horizontal transmission is carried out by cannibalism and through contaminated water (Lightner 1996b; Tang et al. 2003) and vertical transmission via infected eggs (Motte et al. 2003). IHHNV is closely related to densoviruses of the genus Brevidensovirus (Shike et al. 2000). In India, molecular evidence of the presence of IHHNV in *P. monodon* showing slow growth has been observed (Rai et al. 2009). Although there is no effective treatment to cure viral infections in crustaceans, biosecurity measures such as maintaining better water quality, selecting disease-free brood stocks or seeds, augmenting the disease resistance of the host, and hindering the disease transmission process are the major preventative measures that should be ensured to prevent the spread of these diseases.

9.6.2 *Hepatopancreatic Parvo-like Virus (HPV)*

Penaeus monodon densovirus (*Pm*DNV) (formerly hepatopancreatic parvovirus, or HPV) of penaeid shrimp is one of the important shrimp viruses that causes considerable economic loss in shrimp culture all over the world (Flegel 2006). *Pm*DNV, a viral pathogen, belongs to the family Parvoviridae (Bonami et al. 1995). HPV was identified simultaneously in cultured populations of *Penaeus semisulcatus* and *Penaeus merguiensis* in Asia (Lightner and Redman 1985). HPV has been documented in six other species, namely, *P. monodon, P. esculentus, P. indicus, P. chinensis, P. penicillatus*, and *P. vannamei*. The signs of disease in individual shrimp are not specific to HPV and include reduced growth, reduced preening, muscle opacity, and hepatopancreatic atrophy. Cumulative HPV-associated mortality was

reported to be 50–100% after 4–8 weeks in juvenile *P. merguiensis* (Lightner and Redman 1985). HPV infects the epithelial cells of the hepatopancreas. The mode of transmission of HPV is not fully understood, as it has not been transmitted experimentally. Evidence exists that HPV is transmitted vertically from brood stock to progeny and horizontally during the post-larval stages (Brock and Lightner 1990).

HPV in penaeid shrimp causes considerable economic loss in shrimp culture all over the world (Flegel 2006). HPV is highly prevalent in some areas; it was found in 46% of the wild shrimp populations in India (Manjanaik et al. 2005). For farmed *P. monodon*, 31–62% of shrimp were found to be HPV-positive in India and Thailand (Umesha et al. 2003; Flegel et al. 2004). HPV has been found in nearly 100% of the populations of wild *P. monodon* in Africa and also in the wild stock of *P. merguiensis* in New Caledonia (Tang et al. 2008). Two completely sequenced genomes of HPV are available from Thailand (*P. monodon* densovirus) and Australia (*P. merguiensis* densovirus) (Sukhumsirichart et al. 2006; La Fauce et al. 2007). The complete nucleic acid sequence of *Pm*DNV from India revealed that the Indian *Pm*DNV is more closely related to Thai isolates than the other parvoviruses (Safeena et al. 2010).

HPV is a non-enveloped icosahedral virus with an average diameter of 22–24 nm. It has a minus genome with single-stranded DNA due to its distinct genome structure and an approximate genome size of 6 kb. HPV is considered a new member of Densovirinae, a subfamily which is able to infect both vertebrates and invertebrates. HPV-infected penaeid shrimp shows morphological differences in the appearance of the viral inclusion as well as variations in tissue tropism. Hepatopancreatic parvovirus (HPV) infects the hepatopancreas in penaeid shrimp and retards their growth (Phromjai et al. 2002). HPV intranuclear inclusions can be frequently observed in the midgut mucosal epithelium of HPV-infected *P. monodon* post-larvae from Madagascar (Pantoja and Lightner 2000). HPV has a marked tropism for epithelial cells of the hepatopancreas. It was hypothesized that infected shrimp could continuously shed viral particles, almost directly into the feces, after the host cells die and lyse. Catap and Travina (Catap and Traviña 2005) revealed that no HPV infection was produced in adult *P. monodon.*

HPV continues to cause substantial losses in the aquaculture industry in many countries. Control measures such as improvement of environmental conditions, stocking of specific pathogen-free shrimp post-larvae, and augmentation of disease resistance by vaccination using recombinant and RNAi techniques to prevent HPV infection in shrimp are in the experimental stages and are being applied to control HPV infection in the culture systems.

9.6.3 White Tail Disease (WTD)

White tail disease (WTD) is an important viral infection for *M. rosenbergii* due to large-scale mortalities in hatcheries and nurseries, leading to subsequent production losses in many countries such as Taiwan, Thailand, France, India, and the People's

Republic of China (Bonami and Widada 2011). In India, these viral pathogens have been reported in hatcheries and nursery ponds located in Andhra Pradesh and Tamil Nadu (India) (Hameed et al. 2004). White tail disease was also reported in China (Qian et al. 2003), Chinese Taipei (Wang et al. 2006), Thailand (Yoganandhan et al. 2006), Australia (Owens et al. 2009), and Malaysia (Saedi et al. 2012). The causative organisms for WTD were found to be two viral pathogens, namely, *M. rosenbergii* nodavirus (*Mr*NV) and extra-small virus (XSV). The typical gross signs of WTD in infected PL 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, including the head (cephalothorax) muscles, and causing lethargy, abnormal behaviour, anorexia, and weakening of their feeding and swimming abilities. Degeneration of the telson and uropods is observed in severe cases. In all cases, mortality reached 100% within 2–3 days after the first appearance of prawns with whitish muscles (Arcier et al. 1999; 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 the striated muscles of the abdomen, cephalothorax, and intratubular connective tissue of the hepatopancreas. No viral inclusions were observed in epithelial cells of the hepatopancreatic tubules or in midgut mucosal epithelial cells (Arcier et al. 1999).

*Mr*NV is a small, icosahedral, non-enveloped particle, measuring 26–27 nm in diameter. It contains two single-stranded RNAs (RNA1–2.9 kb and RNA2-1.26 kb). Its capsid contains a single polypeptide of 43 kDa. With these characteristics, it is closely related to the *Nodaviridae* family. Later, a second viruslike particle, unusually small (15 nm in diameter) and consequently named XSV (extra small viruslike 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. 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). The 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 its extremely small size and absence of gene-encoding enzymes required for replication, it has been suggested that XSV may be a satellite virus, while *Mr*NV plays the role of a helper virus (Sri Widada and Bonami 2004). Nucleotide sequencing of the *Mr*NV genome indicated that RNA-1 was composed of 3.2 kb nucleotides and that RNA-2 contained 1.17 kb nucleotides.

In horizontal transmission experiments, five developmental stages of *Artemia* were exposed to *Mr*NV and XSV via immersion and oral routes, and it was reported that *Artemia* is capable of transmitting these viruses to *M. rosenbergii* post-larvae and is responsible for causing WTD (Sudhakaran et al. 2006a, b). WTD mainly

affects the PL of *M. rosenbergii* and causes major mortalities in these young animals. Prawn brood stock inoculated with *Mr*NV and XSV by oral or immersion challenge survived without clinical signs of WTD. The survival rate of larvae gradually decreased, and 100% mortality was observed in the post-larvae. Experimental infection of brooders with both *Mr*NV and XSV demonstrated that the vertical route is actually the main mechanism of disease transmission. In the infected brooders, ovarian tissue and fertilized eggs were found to be positive for *Mr*NV/XSV as evidenced by RT-PCR, and a mortality of up to 100% was observed in PL from hatched eggs released from virus-inoculated brooders (Sudhakaran et al. 2007). Control measures such as improvement of environmental conditions, stocking of specific pathogen-free post-larvae, augmentation of disease resistance by immunostimulants, and vaccination using recombinant and RNAi techniques to prevent *Mr*NV/XSV infection.

9.6.4 Infectious Myonecrosis Virus (IMNV)

Infectious myonecrosis (IMNV) is an emerging OIE-listed potential shrimp virus that can cause significant losses in shrimp aquaculture (Nunes et al. 2004). The disease was first reported in cultured *L. vannamei* in Brazil in 2003 (Tang et al. 2005) and later in Indonesia (Senapin et al. 2007) and most recently in India (Sahul Hameed et al. 2017). IMNV belongs to the family Totiviridae and is closely related to *Giardia lamblia* virus. It measures 40 nm in diameter and has an unenveloped icosahedral shape (Lightner et al. 2004; Fauquet et al. 2005; Lightner 2011). The viral genome consists of a single double-stranded RNA of 7,561–8,230 bp (Dantas et al. 2015; Naim et al., 2015; Senapin et al. 2007).

The susceptible hosts for the virus are brown tiger prawn (*Penaeus esculentus*), banana prawn (*P. merguiensis*), and whiteleg shrimp (*P. vannamei*) (OIE 2021). Pathogenicity of this virus, which ranged from 40% to 70% mortality in cultured *L. vannamei*, was reported (Andrade et al. 2008). No other hosts susceptible to IMNV have been reported so far. Clinical signs of the disease are characterized by opaque or discolored skeletal muscle tissues, primarily in the abdominal segment of affected shrimp (Poulos et al. 2006). Histopathology shows that muscle lesions are characterized by myonecrosis, hemocyte infiltration, and the presence of basophilic, cytoplasmic inclusions (Poulos and Lightner 2006; Poulos et al. 2006). The virus shows both horizontal and vertical modes of transmission in *P. vannamei* (Lightner et al. 2004; da Silva et al. 2016).

Control measures to prevent the spread of IMNV infection are good management practices of shrimp farming, unregulated transboundary movement of brood stocks, and post-larvae.

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