Chapter 7 Alarming Viral Pathogens in Shrimp Industry and Nanotechnology



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7.1 Introduction

Shrimp aquaculture has seen immense growth over the past three decades to become a major global food industry which serves the seafood sector based on consumer demand and plays a major role in the economic development of various poor coastal aquaculture communities across the globe. The environmental changes may also serve as a pathway or precondition for the emergence and spread of the disease. Shrimps are taken from their wild environments and fed with artificially composed or synthetic pellet feeds. For higher yield and production, shrimps are stocked in areas with high population density (Ashraf et al. 2011). Due to the changes in water quality, shrimps are exposed to stress and are transported overseas, either as live or as frozen products for consumers. These practices have provided opportunities for increased disease outbreaks due to stress and environmental conditions, exposure of new pathogens to the culture pond, and the rapid transmission of infectious pathogens and the spread of disease. Several scientific domains are participating in this sector to eradicate or control the viral pathogenesis mechanism in the aquaculture industry. Among these, nanotechnology can be used as an efficient method to eradicate the devastating disease-causing pathogenic viruses (WSSV, YSV, and TSV), which collapse the socio-economic value of a country. Nanotechnology is defined as the study and application of functional material devices and systems through the control of matter at the atomic and molecular levels, such as at the nanometer scale,

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and the novel phenomena and properties of matter are exploited at that nanometer scale. There is no comprehensive and concise definition of nanomaterials and nanoparticles. So far, there are various definitions that have been proposed by the government, industry researchers, even private labs, and other research institutes and organizations (Arulmoorthy et al. 2020). The definition of nano is still under debate because there is no proper definition for nano, which is basically said to be based on size. These kinds of nano-based functional devices are used in all the fields of science, such as chemistry, physics, and biology. The whole world is made up of atomic particles, so the effect of nanoscience plays a vital role in bioremediation and bio restoration in aquaculture. Here we are going to discuss the viral diseases that have majorly affected the shrimp culture industry over the past two decades. Some of the diseases are addressed in Table 7.1.

7.2 White Spot Syndrome

White spot syndrome virus is a type of virus within the genus Whispovirus in the Nimaviridae family. Basically, WSSV virions are rod- or elliptical-shaped, symmetrical, and approximately 80-120 nm in diameter and 250-380 nm in length as per measurement. It is noted that there are 39 structural proteins at the end of the flagellum like appendages on virions. The virulence of the WSSV shows differences among diverse isolate varieties, and it has a different-sized circular double-stranded DNA genome. WSSV, formerly known as systemic mesodermal and ectodermal baculovirus, is a nonoccluded baculovirus-like agent infecting many penaeid species, first examined in P. japonicus. Within 2-5 days of time, WSSV has the ability to cause up to 100% mortality in commercial shrimp farms, and as a result, the shrimp aquaculture industry faces a huge economic loss across the globe. WSSV mostly attacks shrimps at the postlarval stage. However, disease occurrence is even seen in growing juvenile shrimp of all ages and sizes, but mostly from 1 to 3 months after stocking in grow-out ponds. WSSV outbreak can occur irrespective of farming system, stocking density, water quality, and salinity. The subsequent source of WSSV infection is dead or infected shrimps; cannibalism is said to be one of the main reasons for the transmission of the virus. Even infected pond water acts as the waterborne route (Sivakamavalli and Vaseeharan, 2014). In shrimp farms, the major source of infection is due to the infected spawners and postlarvae (PL) from the infected broodstocks of hatcheries. The vertical transmission of WSSV by trans-ovum also acts as a major reason for the transmission of the disease. Rotifers, marine molluscs, polychaete worms, Artemia salina, Ephydridae insect larvae, and sea slaters (Isopoda) are the major disease-carrying vectors of the white spot syndrome virus.

7.3 Symptoms

- In moribund shrimp, gross signs are seen along with rapid mortality.
- Lethargy, black gills, anorexia, broken antennae.

Table 7.1 Common viral dise	eases and its causative agents in d	different shrimp	o species			
Disease	Virus	Abbreviation	Genome	Family	Genus	Species affected
White spot syndrome	White spot syndrome virus	WSSV	dsDNA	Nimaviridae	Whispovirus	All farmed marine penaeid shrimp
						species
Infectious myonecrosis	Infectious myonecrosis virus	IMNV	dsRNA	Totiviridae	Giardiavirus	P. vannamei
White tail disease	Macrobrachium rosenbergii	MRNV	(+)	Nodaviridae	Related to Alphanodavirus	P. vannamei
	nodavirus		ssRNA		and Betanodavirus	
Infectious hypodermal and	Infectious hypodermal and	VNHHI	ssDNA	Parvoviridae	Brevidensovirus	P. monodon
hematopoietic necrosis	hematopoietic necrosis virus					P. vannamei
						P. stylirostris
Taura syndrome	Taura syndrome virus	TSV	(+)	Discistro-	Cripavirus	Penaeus vannamei
			ssRNA	viridae		

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Fig. 7.1 WSSV-infected carapace of a shrimp

- Swollen branchiostegites.
- On the cuticle of the cephalothorax or carapace, white deposits of calcium can be seen.
- Display expanded chromatophores and cephalothorax cuticles.
- Along with white spots all over the body surface (Fig. 7.1).

The target tissues or cells for the white spot syndrome virus are of mesodermal and ectodermal origin: the cuticular epithelium, lymphoid organs, the heart, haematopoietic tissue, the cuticular epidermis, subcuticular tissue and other connective tissue, the antennal gland, and the hindgut. In chronic cases, the nervous system and compound eyes are infected, and the enlarged haemal sinuses and interstitial spaces are caused by the diseased lymphoid organ of shrimp, which will result in a hypertrophied yellowish hepatopancreas. Sometimes an empty gut also indicates the disease occurrence, along with cuticular epiboint fouling and lymphoid organ swelling. Marine penaeid shrimps are the major victims for white spot syndrome virus which also includes marine, brackish water and freshwater prawns, crayfish, crabs, shrimps and lobsters. *Macrobrachium rosenbergii, Penaeus monodon, Litopenaeus vannamei, L. stylirostris, M. japonicus, F. indicus, Procambarus clarkia*, and *P. leniusculus* are some of the common examples of farmed aquatic animals affected by WSSV.

7.4 Control and Prevention

• There have been no vaccines developed against WSSV. In recent studies, nanotechnology has a really good effect when compared to other methodologies.

- The pathogenicity of WSSV is decreased at low temperatures $(12 \pm 2 \text{ °C})$, and also it inhibits the mortality rate in crayfish and shrimp.
- Certain bio-components, such as polysaccharides bound with sulfate, and microalgae cell walls, can be used as immunostimulants against WSSV in shrimp.
- It can be prevented or avoided by stocking season-specific pathogen-free (SPF) larvae or PCR-tested larvae in the culture system.
- In *P. monodon*, the usage of β -1,3-glucan as a feed mix along with shrimp feed effectively improves the immunity and survival.
- Certain probiotics of *Bacillus* species and *Dunaliella* extracts show some reasonable resistance in shrimp against WSSV infection.
- The epizootics can be reduced by good farming and farm management practices, along with the control of environmental variables such as PH, temperature, salinity, and other factors, which play a vital role in the genetic improvement of the penaeid species of shrimp.

Several approaches might be undertaken through molecular biology approaches, but at the same time, the rapid development of nanotechniques is an unavoidable arena in every platform to eradicate and control scientific problems. On this connection, various nanoparticles, nanocomposites, nanospheres, nanorods, and nanocubes have been applied in bacterial inhibitory mechanisms in agriculture as well as aquaculture platforms. However, few viral infections wouldn't be possible to eradicate completely from the culturing sectors, and at the same time, we can control the viral infections through different nanoformulations.

7.5 Infectious Myonecrosis

Penaeid shrimps are the main victims of the infectious myonecrosis virus (IMNV). So far, the IMN has been reported only in shrimps from rearing ponds, but not in the wild populations due to the lower density of shrimps in the marine environment. Litopenaeus vannamei (Pacific whiteleg shrimp) and Farfantepenaeus subtilis (Southern brown shrimp) are easily prone to the IMNV, and this makes a huge economic loss for a country in the food sector. In P. monodon, due to IMN infection, there have been no reports of death; even though there are no death reports for P. monodon, it can act as a potential carrier of the virus. When compared to other virulent shrimp viruses, the IMNV does not affect any vital organs because its target tissue is skeletal muscles, so, therefore, IMNV is not that fatal. The damaged muscle tissues can be repaired within the early five stages of infection (Tang and Kotov 2005). Various studies have been done to understand the source of potential carriers of the IMNV and (da Silva et al. 2015) reported that a zooplankton named Artemia salina, which is used as a live feed, acts as the major carrier of IMNV infection. But the ones which fed on infected artemia do not show any mass mortality. This may be because they act as a source of IMNV only in grow-out culture ponds. Certain worm types such as polychaete worms and blood worms showed a positive response to IMNV infection. The presence of the virus may be due to the ingestion of contaminated tissues or water from the infected area. The IMNV has some peculiar and

specific characteristics. These specific characteristics and behaviors made this double-stranded RNA virus one of the most dangerous shrimp industry viruses because it is a stress-dependent virus.

7.6 White Tail Disease

The most extensively cultivated shrimp species was Litopenaeus vannamei (L. vannamei) across the coastal communities of the world. This is because of its high yield and high market value. The emergence of various viral diseases such as WSSV, YHD, TSV, and alpha- and betanodavirus is due to the continual expansion and intensification of shrimp farming. Among the abovementioned diseases, IMNV and alpha- and betanodavirus are the major causative pathogens for the emergence of white tail disease (WTD), as well as *Macrobrachium rosenbergii* nodavirus (MrNV) identified in freshwater prawns (Naveenkumar et al. 2020). The target tissue for both of the viruses are primarily skeletal muscles, which results in gross signs such as white or opaque tail and histopathologically shows muscle necrosis and changes in lymphoid organs as spheroids, moreover in all Penaeid shrimps. In recent studies, it has been identified that a highly virulent Vibrio harvevi strain HLB0905 is the causative agent for the white tail disease. This can be examined through microscopical morphology analysis, genome sequence analysis, and bacterial isolation. Interestingly, in L. vannamei, the particular strain of V. harvevi not only causes "white tail disease" (WTD) but also was nonluminescent in nature (Zhou et al. 2012).

7.7 Infectious Hypodermal and Hematopoietic Necrosis

This is a viral disease which mainly infects the penaeid shrimp variety and may cause up to 90% of mass mortality in culture ponds as well as in wild environments in the Pacific region. Infectious hypodermal and hematopoietic necrosis virus (IHHNV) can be seen in the population of *Penaeus stylirostris* and in *Litopenaeus vannamei*. Recent studies made by the shrimp-farming industries and the researchers of aquaculture have developed several broodstocks which are resistant to the IHHNV in both P. stylirostris and L. vannamei. Decapod pestylhamaparvovirus is the causative pathogen of IHHN, which is notably a single-stranded DNA virus and also the smallest known virus at 22 nm that infects shrimp. The virus has been classified as P. stylirostris hamaparvovirus (Natrah et al. 2011). The replication of the virus is through the host cell cytoplasm, so it can be even classified as a picornavirus. The symptoms of IHHN infection are reduced food consumption, increased mortality rate of 80-90% in the juveniles grown in high-density and raceway cultures, and even cannibalism in culture systems of penaeid shrimp. Some of the species of Penaeidae family survive IHHNV infection, but they carry the virus for their whole life and transfer it to the progeny and other populations by vertical or horizontal transmission. IHHNV infection is also known as runt deformity syndrome (RDS) in L. vannamei.

7.8 Taura Syndrome

Taura syndrome virus (TSV) is a virus which acts as the causative pathogen for taura syndrome (TS) infection. Litopenaeus vannamei and P. stylirostris are the common host species of the TS virus and are easily susceptible to TS infection, whereas TS can be classified into three distinct phases: acute, transitional, and chronic. In the acute phase, a histological section of epithelium and cuticular epithelium shows lesions. But in the transition and chronic phases of the disease, there are no signs of lesions in the epithelium, and the TSV infection can be detected only by molecular and antibody-based methods. By good farming practices, an individual shrimp can be protected from chronic TSV infections which may persist for the whole lifespan. The cuticular epithelium of the exoskeleton, the gut region, the gills, and the appendages are the major sites of TS virus replication, and initially, it infects these regions. The histological sections of gastroenteric organs, cardiac and striated muscles, the ventral nerve cord, and its branches and ganglia do not show any signs of TSV infection and are usually negative for TSV by ISH (in situ hybridization). Pale reddish discoloration on the tail fan and pleopods is seen in the penaeid shrimp, postlarvae, or older shrimps of Penaeus vannamei, which is also known as red tail disease. The expansion of the red chromatophores in the cuticular epithelium is the reason for the color change and the peppered appearance. The reddish discoloration of the tail fan and uropods has rough edges on the cuticular epithelium. These are the symptoms of the acute phase of Taura syndrome, which shows lesions.

7.9 Yellow Head Disease (YHD)

The causative pathogen for Yellow head disease (YHD) is Yellow head virus (YHV). YHV is an ssRNA virus which is closely associated with the families Coronaviridae and Arteriviridae. Gill-associated virus (GAV) is closely related to the YH virus, which comes under the genus Okavirus and has a wide range of hosts. Naturally, the infections occur particularly in P. monodon, but infections are also reported in P. japonicus, L. vannamei, P. setiferus, and P. stylirostris. Penaeus merguiensis and Palaemon styliferus are resistant to YH disease, but they act as carriers of viral pathogens. Euphausia spp. (krill), Acetes spp., and other small shrimps are also reported to act as carriers of YH viruses. YHD was reported in Thailand for the whole year of 1999. Aggregation of diseased shrimps can be seen at the very edge of the pond. Diagnosis of Yellow head disease can be confirmed by the following symptoms: discoloration of the hepatopancreas, which results in the yellowish appearance of the cephalothorax; this is the reason for the name of the disease. The overall appearance of the shrimp becomes abnormally paler than usual, and it affects many tissues such as gills, lymphoid organs, hemocytes, and connective tissue. Histopathology: degenerative changes in nuclei and presence of cytoplasmic basophilic inclusion bodies. Older shrimp and postlarvae (PL) of 15–25 days are infected, while postlarvae of more than 15 days appear resistant. Mass mortalities of up to 100% are observed within 3-5 days (Hasson et al. 1995).

7.10 Nanotechnology in Detection and Disease Management in Shrimp

Nanoarrays, protein arrays, and nanopores are used in the detection of viruses. These are nanomaterials which can be used in the management of viral diseases in shrimp. Many researchers are trying to use nanoparticles as nano-based sensors and nano-vaccines to prevent disease outbreaks and run mortality in growing-out ponds (Flegel, 2019). A simple and rapid immune-chromatographic test-strip kit used for WSSV detection is being developed by various researchers and organizations with gold nanoparticles (AuNPs) conjugated with monoclonal antibody W29 as the detector antibody (Khosravi-Katuli et al. 2017). In this method, rabbit antirecombinant protein antibody is used in permutation with W28 monoclonal antibody as an arrest complex in the test line and goat anti-mouse IgG antibody (GAM) as an arrest antibody in the control line. This method is demonstrated using chromatography through the nitrocellulose membrane. While the test is four times less sensitive than the dot blot technique and is much less sensitive than single-step PCR, it was suggested to test samples from the individual as well as combined shrimp samples to validate high probabilities of WSSV infection and disease outbreaks. The working mechanism of this kit is simple, easily approachable, and very convenient for obtaining immediate results without the help of hi-fi tools or technicians with previously learned skills (Rather et al. 2011).

A rapid and label-free detection of the WSSV with a surface plasmon resonance (SPR) device based on gold films equipped with electroless plating was developed by Lei et al. (2008). The electroless-plated gold films were modified with single chain fragment variable (scFv) antibody molecules and were used in combination with a highly sensitive SPR binding between a WSSV sample and the anti-WSSV scFv antibody pre-immobilized against the sensor surface device for detection of the virus present in different concentrations in shrimp hemolymph matrix. The method could detect the application of WSSV as low as 2.5 ng/mL in shrimp hemolymph, so enzyme-linked immunosorbent assays cannot be done due to the lower CFU levels (Govindaraju et al. 2019). Though time-consuming, the method is costeffective and highly reproducible, which could help increase sensors performanceprice ratio of SPR sensors, suggesting that it can be used widely in the fabrication of SPR sensor substrates. To develop a prototype for the development of a simple and cheap diagnostic tool for in-field testing in farms, Thiruppathiraja et al. (2011) used alkaline phosphatase along with AuNPs, which is used as a primary vaccine for WSSV infection. Also they have successfully enhanced the immunity through conventional methods, where visually it can be seen up to 1 ng/mL of purified WSSV (Thiruppathiraja et al. 2011). However, though protein-based immunodetection methods are easier to perform, they lack the sensitivity appropriate to adequate signal amplification, which is a major impediment to taking this technique forward for wider application. Several genetic diseases can be treated by the development of novel carrier systems for gene delivery; this can be done by nanotechnology by means of nanoparticles. Certain approaches utilize DNA complexes containing proteins, lipids, polypeptide carriers, as well as nanoparticles with ligands that are capable of targeting specific DNA complexes to the cell-surface receptors on target cell sites and directing intracellular trafficking and delivery of DNA towards the nucleus. One among those is the formation of complexes between chitosan and DNA polyplexes for oral gene therapy applications. Nanoparticles loaded with plasmid DNA could serve as a proficient gene delivery system due to their quick movement to the cytoplasmic compartment from the degradative endo-lysosomal compartment. Repeated gene expressions can be seen due to the constant discharge rate of DNA. Studies on the protective efficacy of the oral delivery of a DNA construct containing the VP28 gene of WSSV encapsulated in chitosan nanoparticles in P. monodon showed 50% survival even after 30 days, while the control registered 100% mortality (Govindaraju et al. 2019).



7.11 Using AgNPs Against Shrimp Viral Diseases

It is proven that various types of pathogens infecting humans can be treated with nanoparticles, especially AgNPs, but still the effects of AgNPs on shrimp diseases have limited information (Boverhof et al. 2015). The source of evidences from in vivo studies shows that AgNPs have the potential for antimicrobial activity against certain shrimp diseases like WSSV and bacterial diseases caused by Vibrio parahemolvticus and other Vibrio species. WSSV is a vigorous strain of virus which rapidly spreads over the culture area and causes a complete spread of white spot disease on the shrimp ponds in a particular area, which makes it an alarming danger for farms around the globe. Vibriosis, brown spot disease, and acute hepatopancreatic necrosis syndrome (AHPNS) are some of the bacterial diseases caused by Vibrio. These bacterial types are ubiquitous in all coastal and brackish water ecosystems in all parts of the world. The abovementioned disease causes considerably huge economic losses in the seafood sector in all the coastal communities, farms, and hatcheries across the globe. Recent studies show that viral and bacterial diseases of shrimp can be rectified by the application of nanoparticles, these applications can be used for therapeutic and prophylactic purposes. The administration of drugs such as vaccines and other medications before the disease outbreak is known as a prophylactic application, whereas the application of drugs for an ongoing active disease in growing-out shrimp ponds is known as a therapeutic application. In marine shrimps, vibrio species that are pathogenic against them are found to be inhibited by the AgNPs synthesized through chemical, biological, and even biochemical methods (Shaalan et al. 2016). In Penaeidae shrimp family like Fenneropenaeus indicus, P. monodon, and L. vannamei, it is proven that to some extent nanoparticles prevent certain diseases of shrimp caused by Vibrio species, which include vibriosis and APHND in postlarvae of L. vannamei. In those cases, the prophylactic effects of nanoparticles translate to a higher survival rate of individuals exposed to bacteria after AgNPs administration over a period of time, in contrast to untreated animals that presented the expected elevated mortalities (Camacho-Jiménez et al. 2020).

7.12 Conclusion

Nanotechnology brings diagnostic applications on various platforms, with diagnostic approaches including viral diseases in agriculture, aquaculture, and shrimp disease management. Nanocarriers can be used to improve stability, increase immunostability, and also target specific immune cells. They are used in the formulation of antigens for delivery to specific target sites, and there are lots of other adjuvant advantages. In the near future, many products based on nanotechnology applications, including the detection of infection based on nanomaterials and disease management, will be on field trials, and promising results have been seen so far. Disease or infection detection can be done by the abovementioned products, which can be rolled out in the market for field trials. Even treatment and prevention of viral pathogens such as WSSV, IMNV, IHHNV, YHD, and TSV can be done using nanotechnology and its nanomaterials. The rapid development of nanotechnology in aquaculture, especially in the shrimp industry, can revolutionize the diagnosis and treatment of shrimp disease. Nowadays, nanosensors in the pond environment are used to detect variations in water, such as mineral deficiency, mortality, ammonia (sludge) levels, and even malicious disease outbreaks. To prevent environmental and disease stresses, nano-based functional feeds are developed. Several other ideologies include the removal of pollutants from aquaculture pond systems and the enhancement of finfish and shellfish growth for sustainable aquaculture farming practices.

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