



ISSN: 2456-2912

VET 2024; SP-9(2): 352-356

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Received: 19-01-2024

Accepted: 24-02-2024

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## A most lethal pathogen of shrimp in aquaculture: Systematic review

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### Abstract

Disease outbreaks are more prominent in aquaculture throughout the world due to favourable condition which subsequently reduce industry's productivity, profitability, and long-term viability in global aquaculture production. One of the most common diseases in Asia, WSSV causes most dangerous to shrimp aquaculture. Vaccinations and other techniques have shown to be an effective pathogen-prevention strategy against the pathogens. For example, immunizations and other preventive measures that are developed to shield shrimp aquaculture against various viral diseases. But developing such preventive methods as quickly as needed would be difficult because diseases and germs infrequently. This review aims to collect information on the host ranges, immunity to the WSSV virus, genome size, morphology of the virus, pathological changes, and prevention and management procedures that have been used to develop a successful aquaculture.

**Keywords:** Pathways, probiotics, mortality, RNAi, monovalent

### Introduction

With worldwide aquaculture production growth rate were 2015 2022 was 8.1%, which make aquaculture industry fastest animals food producing sectors and becoming a significant source cheaper proteins diet. In 2020, fish production was recorded at 179 million tons worldwide, with an anticipated US\$ 401 billion as the overall first sale value. Over the past 30 years, Asia has produced 89% of the world's total in volume terms, making it a major producer globally. China is the world's largest producer of fish, producing 1.8 million tons annually. Aquaculture is one of the food animal production industries with the fastest rate of growth, thanks to its use of advanced farming techniques.

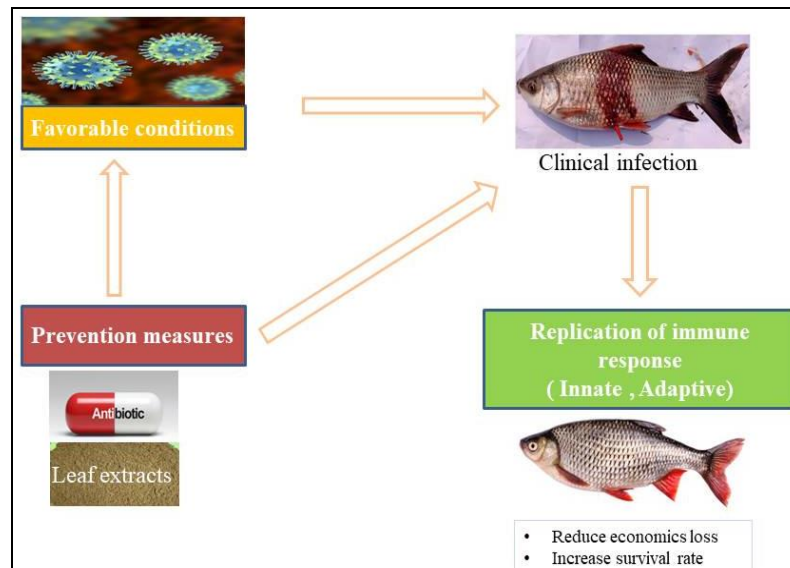
Aquaculture methods that are successful rely on biological, chemical, and physical elements such water as well as the health of the fish raised there (Prabu *et al.*, 2019) [28]. High fish farming productivity results from intensifying and diversifying aquaculture; but, uncontrolled transboundary migrations of aquatic animals and animal products throughout the world can cause serious disease outbreaks. Fish under the intensive culture method were cultivated in conditions of high stocking density, which put them under stress. Stress is brought on by bacteria, viruses, parasites, and fungus, among other abiotic and biotic variables such as low water quality parameters and ambient temperature. Nevertheless the advancements, the aquaculture sector has suffered significant financial losses due to the advent of infectious diseases such as the white spot syndrome virus (WSSV). The virus is extremely infectious and can kill 100% of shrimp within three to ten days of an epidemic outbreak in the farming system. The projected global economic loss resulting from WSSV since its inception in 1991 is between 8 and 15 billion USD (Dey *et al.*, 2020) [6].

### Structure, shape, morphology and genome characteristic of WSSV

The virus known as white spot syndrome is bacilliform, non-occluded, enveloped, and its enveloped virions have a maximum width of 70 nm and a length of 210–380 nm (Chou *et al.*, 1995; Flegel and Alday-Sanz, 1998; Li *et al.*, 2005; Rajendran *et al.*, 2003) [42, 8, 23, 29]. According to research on the WSSV, different viral particles have different genome sizes. On average, the genome spans approximately 300 Kilobases.

ATG initiation codons are found in between 531 and 684 open reading frames (ORFs) in the WSSV genome, according to studies on sequence analysis (Wang *et al.*, 1995; Yang *et al.*, 2001) [42, 45]. Occasionally, a tail-like appendage at one end of the WSSV virion can be seen on electron micrographs stained negatively. As described by Durand *et al.* (1997) [7] and Nadala and Loh (1998) [26], the nucleocapsid is a stacked

ring structure inside the envelope that resembles a crosshatch. It is composed of globular protein subunits with a diameter of 10 nm grouped in 14–15 vertical striations spaced every 22 nm along the long axis. About 21–29% of these ORFs have been shown to encode WSSV proteins or to share their identity with other known proteins.



### Clinical manifestation, host range and pathological changes induce by WSSV

White spot disease (WSD), which affects several economically significant decapod species, is caused by WSSV. In Asian shrimp farms, WSSV was initially discovered in 1921 (Inouye *et al.*, 1994) [16]. It was then reported in several other nations throughout the world (Karunasagar *et al.*, 1997; Stentiford and Lightner, 2011) [17, 38]. Clinical symptoms of the condition might include reddish-brown body discoloration, lethargy, decreased eating, and increased mortality in addition to white patches on the exoskeleton. WSSV has a broad spectrum of hosts or carriers, including over 93 species of arthropods and even polychaetes, where WSSV accumulation has been seen in their digestive tracts, in addition to shrimp (Flegel, 1997; Sánchez-Paz, 2010) [9, 32]. Durand *et al.* (1997) [7] and Wang *et al.* (2000) [43] have reported that hypertrophied nuclei displaying marginated chromatin and amphophilic intranuclear inclusions are suggestive of WSSV infection. These intranuclear inclusions are notably larger and different from Cowdry A-type inclusions found in infectious hypodermal and hemopoietic necrosis viruses. Over time, infected nuclei expand and become more basophilic.

### Immunity against the WSSV infection in shrimp

Generally, invertebrates are having cellular as well as humoral immunity to fight against the pathogenic microorganism and give protection from these pathogens. Plants, arthropods, and mammals have all evolved to use RNA interference (RNAi) as a post-transcriptional gene regulatory mechanism. It is the regulation of target gene expression by means of chromatin remodeling, translation suppression, and mRNA instability (Geley and Muller, 2004) [11]. There are number of proteins (Drosha, Pasha, Dicer1 and Ago1) with miRNA biogenesis in shrimps. Till now, more than 40 viral miRNAs encoded by WSSV reported viral infected cells. A growing volume of research indicates that the main players in the shrimp canonical miRNA synthesis

pathway are involved in a number of viral infections, including occasionally WSSV. PmAgo, a *P. monodon* Ago1 protein with over 80% sequence homology with insect Ago1 homologs, was specifically stimulated to rise two- to three-fold (YHV) during the early stages of yellow head virus infection (Unajak *et al.*, 2006) [40]. Furthermore, in order to promote viral infection, WSSV miRNAs target virus genes. He and colleagues demonstrated that WSSV-miR-66 targeted the *wsv094* and *wsv177* genes during the early stages of WSSV infection, whereas WSSV-miR-68 targeted the *wsv248* and *wsv309* genes (He *et al.*, 2014) [13]. Infected cells containing virus-derived siRNAs (vsiRNAs) suggest that RNA interference (RNAi) is a natural antiviral immunity mechanism in plants and insects. vsiRNAs serve as defense mechanism specificity determinants as well as a molecular marker for the induction of natural antiviral RNAi.

Numerous PPRs are recognized in vertebrates; these include nucleic acid-based components like TLR9, AIM2, and cGAS, which identify RNA in the form of dsDNA, other TLRs, RIG1, and so forth (Barrat *et al.*, 2016; Hornung *et al.*, 2014) [3, 14]. According to a report (Robalino *et al.* 2004) [31], researchers found that different unrelated dsRNAs could provide shrimp with sequence-independent antiviral protection against WSSV and TSV. The suggested that innate immunity is being activated as a component of the system. According to a study by Li *et al.*, (2015) [21], the promoter of the secreted interferon-like cytokine *Vago4* interacts directly with the IRF-like transcription factor in shrimps, activating the JAK/STAT signaling pathway.

*Drosophila* Dicer2 and shrimp Dicer2 are nucleic acid receptors just like RIG-I in mammals and which is up regulated against the challenge WSSV infection in shrimp (Chen *et al.* 2011) [5]. Apoptosis is a fundamental kind of innate host defense that is thought to have been evolutionarily conserved across animal species. It plays a significant role throughout development and homeostasis which is activation of enzyme caspase cascades in the organism. According to MacKenzie and Clark (2012) [25], the members of a family of

highly conserved cysteine-dependent aspartate-specific proteases, caspases are essential for regulating and performing apoptosis and have done so throughout evolution. With the help of other adaptor molecules, cytochrome c can cause the cleavage and activation of initiator caspase-9. Through the cleavage of internal mitochondrial route and the extrinsic death receptor pathway can be connected (Luo *et al.*, 1998) [24].

Shrimp's humoral immunity to WSSV infection has also been thoroughly studied. It includes the ProPO system's ability to fight infection, the Toll pathway, the IMD pathway, and interference with the JAK/STAT pathway. Shrimps' exceptional expression against *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, and *Staphylococcus aureus* bacterial infections suggests that both Gram-positive and Gram-negative bacteria are likely to be the cause of the Toll and IMD pathway activation in shrimps (Li and Xiang, 2013) [22]. According to research, shrimp Tolls function as pattern recognition receptors through mutual interaction, similar to human TLRs. They can identify microbially generated products like LPS, PGN, and CpG ODN (Sun *et al.*, 2017) [39]. It is still not entirely clear how the shrimp IMD pathway is activated and regulated. After bacterial infection, Relish is generally the target of signaling directed by the IMD pathway. To activate Relish, phosphorylation and cleavage are needed to eliminate the C-terminal I $\kappa$ B-like domain. A key component of the nucleic acid-induced nonspecific antiviral signaling system in shrimps is the JAK/STAT pathway. The knockdown of LvJAK from *L. vannamei* increases the viral burden and death rate subsequent to WSSV infection (Song *et al.*, 2015) [36]. WSSV has created several strategies to disrupt the host's JAK/STAT pathway and encourage the expression of its genes. The prophenoloxidase activating system (proPO system) controls melanization in a range of invertebrates, which is thought to be an essential component of host defense in response to pathogenic infection. Thus far, the melanization/proPO system in shrimps has been found to be composed of prophenoloxidase 1 (proPO1), proPO2, proPO-activating enzyme 1 (proPPAE1), and proPPAE2 from *L. vannamei* and *P. monodon* (Sritunyalucksana *et al.*, 1999; Lai

*et al.*, 2005; Charoensapsri *et al.*, 2011) [37, 19, 4].

### Control measure and prevention methods of WSSV in aquaculture

There are various methods to control the viral infection in shrimp culture which include probiotics, vaccines, peptides, immunostimulant, prebiotics, RNAi, leaf extracts etc. However, due to the complex ecosystem of pond, it is difficult to control infection fully and to protect the aquatic animals. Plasmid-loaded nanoparticles have the potential to be an effective gene delivery mechanism because of their rapid transition from the cytoplasmic compartment to the degradative endo-lysosomal compartment (Panyam and Labhasetwar, 2003) [27]. As per the investigation done by Rajeshkumar *et al.* (2009) [30] found that the protective efficacy of encapsulating the VP28 gene of WSSV in chitosan nanoparticles by oral administration in *P. monodon* revealed 50% survival even after 30 days, whereas the control group experienced 100% mortality. There is study claimed that the vaccine can be injected via nanoparticles as carrier and no abnormality caused by this particle in shrimp. Five main WSSV structural genes—VP28, VP26, VP24, VP19, and VP15—were targeted in the study conducted by Sarathi *et al.* (2008) [2, 18] in order to synthesize equivalent dsRNA since the proteins that these genes express is widely distributed inside the WSSV and are essential to the infection process. Sivakumar *et al.* (2016) [35] tested 388 marine bioactive compounds against the envelope protein VP28 using MD simulations and flexible molecular docking. The lead candidates were chosen according to the binding free energy (DG<sub>b</sub>) of MM/PBSA. Their results showed that the compounds with the highest binding free energies were Lindgomycin and Astropectenol B, at about 31.031 KJ/mol and 40.453 KJ/mol, respectively. Several studies suggest that adding seaweed and its extracts to food may be a potential treatment for WSSV infection in shrimp. Schleder *et al.* (2020) [33] recently investigated the effect of combining the brown seaweeds *Sargassum filipendula* and *Undaria pinnatifida* on resistance to WSSV-infected *L. vannamei*.

**Table 1:** Overview of WSSV infection in shrimp aquaculture

Shrimp species	Mortality (control)	Survival rate	Immunological parameters	Prevention	Country	Ref.
<i>P. monodon</i>	100%	50 -86%	prophenoloxidase, superoxide dismutase and superoxide anion increased significantly	DNA construct with chitosan nanoparticles	India	Rajeshkumar <i>et al.</i> , 2009 [30]
<i>L. vannamei</i>	78.1%	68- 70%	Increased lipid metabolism, and modulation in AMPs	Sea weed extract	Brazil	Schleder <i>et al.</i> , 2020 [33]
<i>L. vannamei</i>	-	-	Increased survival	dsRNA	USA	Robalino <i>et al.</i> , 2004 [31]
<i>L. vannamei</i>	100%	100%	Increased haemocytes	Probiotics	Mexico	Leyva-Madrigal <i>et al.</i> , 2011 [20]
<i>L. vannamei</i>	100%	Increased (35.5% in 10 <sup>9</sup> CFU/g group)	The expression of five immune genes—proPO, SOD, LvToll, hemocyanin, and STAT—increased.	Probiotics	Thailand	Wanna <i>et al.</i> , 2023 [44]
<i>P. monodon</i>	100%	68%	SOD CAT decreased	Probiotics	Korea	Sekar <i>et al.</i> , 2016 [34]
<i>L. vannamei</i>	100%	83%	proPO, PE and LGBP gene increased significantly	Probiotics	China	Fu <i>et al.</i> , 2011 [10]
<i>P. monodon</i>	100%	100%	NA	Plant extract (2%)	India	Balasubramanian <i>et al.</i> , 2008 [2]
<i>P. vannamei</i>	100%	65-85%	Total hemocyte count, glucose, oxyhemocyanin, SOD, phenoloxidase activity, and total protein	Olive leaf extract	Iran	Gholamhosseini <i>et al.</i> , 2020 [12]
<i>P. monodon</i>	100%	100%	NA	extract of <i>Cynodon dactylon</i>	Bangladesh	Howlader <i>et al.</i> , 2020 [15]
<i>P. monodon</i>	100%	67-83%	NA	Whole cell vaccine	Philippines	Amar <i>et al.</i> , 2011 [1]
<i>P. monodon</i>	100%	71.2±3.13%	NA	Whole cell vaccine	Philippines	Amar <i>et al.</i> , 2011 [1]
<i>P. monodon</i>	100%	56.6–90%	prophenoloxidase and SOD increased	DNA vaccines	India	Kumar <i>et al.</i> , 2008 [18]



## Conclusion

Due to the mortality in shrimp aquaculture caused by the viral diseases leads serious economic loss to the farmers of particular country which subsequently effects production. Many South East Asia countries are mostly impacted by the WSSV disease particularly in shrimp culture. So, it is better to understand host-pathogen interaction, pathogenesis, virus size, genome, replication cycles and immune response generated in the shrimp against the disease. This can help to develop proactive disease management strategies in combating this pathogen. Contagious nature of WSSV leads the disease difficult to treat or cure in the culture systems. In order to better understand WSSV and other aquaculture diseases, this review has attempted to gather crucial data on host ranges, immunity, genome size, morphology, pathological changes, and management and prevention techniques. This will create and implement long-term solutions that protect aquaculture productivity and viability in the face of disease challenges, more research, cooperation, and innovation are needed in the future.

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