White Spot Syndrome Virus-A Threat To Sustainable Shrimp Cultivation

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Abstract
White Spot Syndrome Virus (WSSV) causes a serious contagious disease capable of causing 100% mortality in shrimps and many other crustaceans. Since the first outbreak of this disease in China in 1992, it has gradually spread to all parts of the world. This is the most devastating crustacean disease which threatens aquaculture, globally causing enormous economic losses. WSSV is an enveloped bacilliform rod shaped virus with a large genome of 300 kb ds DNA and has been assigned the genus-Whispovirus in the family Nimaviridae. Symptoms of the disease are manifested as white round spots on cuticular epithelium and carapace with reddish discoloration on the body and appendages of shrimps. The virus causes severe pathological damage to gills, stomach, heart, lymphoid organs, hematopoietic and muscle tissues. Diagnosis of WSSV is based on histopathological, immunological and molecular tests. The virus has a very wide host range and transmission is by both vertical (from infected brood stock to post larvae) and horizontal (by carrier animals, cannibalism and predation of infected organisms) routes. This review details the geographical appearance cum establishment, viral characteristics, genomic properties, pathological, transmission and epidemiological features of this disease. This paper also outlines the ways to prevent and manage the disease burden posed by WSSV to aquaculture. The latest technological control measures deploying vaccines, RNAi and CRISPR-Cas approaches have also been discussed.

Keywords: Shrimp farming, White spot disease, WSSV, Histopathology, Nested-PCR, Prevention, Biosecurity, Disease Control

Introduction

Aquaculture, which is ushering in a Blue Revolution, has the promise of greatly augmenting income and assuring the availability of affordable quality protein to the poor in the developing world. Though aquaculture is one of the world's fastest growing industry but viral disease outbreaks in shrimp have caused serious economic losses and threatened its sustainability.

White Spot Syndrome Virus (WSSV) causes the most dreadful and serious disease of shrimps, worldwide. This virus is highly lethal to all the cultivated shrimp species like tiger shrimp (*Penaeus monodon*), Pacific White Leg Shrimp (*Litopenaeus vannamei*), Indian Shrimp (*Penaeus indicus*), Japanese Shrimp (*Penaeus japonicus*), etc. Its acute infection can cause 100 % mortality in shrimp farms within 3-10 days.

The southern provinces of Mainland China and northern counties of Taiwan , for the first time reported the outbreak of WSSV in 1992 (Chou et al. 1995). The isolation of the causative virus was done during an outbreak in Japan in late 1993 (Inouye et al.1994). Subsequently, WSSV has spread to all shrimp farming countries of the world (Flegel,1997). India reported its first WSSV case in 1994 along the east coast which then quickly spread to the west coast and then to other parts of the country (Karunasagar et al. 1997 , 1998; Mohan et al. 1998 ; Shankar and Mohan, 1998). Losses from the 1992 and 1993 outbreaks throughout Asia were valued at ~US$6 billion (Lightner 2011).

The first diagnosed case of WSSV in the Americas occurred in 1995 in a South Texas shrimp farm, and it was suggested that the most probable route for its introduction was through an Asian imported frozen-bait shrimp commodity (Hasson et al . 2006). In February 1999, the virus caused massive mortalities in some farms in Ecuador (Galaviz-Silva 2004), while the most recent outbreak in an area with WSSV-free status according to the World Organization for Animal Health (OIE) criteria, occurred in Brazil in 2005. Almost all the shrimp growing countries have now reported this disease and it has now assumed a pandemic status.

Clinical Signs

The disease is so named as it exhibits white spots in infection. WSSV-infected shrimp may rapidly develop white spots (ranging from 0.5 – 3.0 mm in diameter) on the exoskeleton, appendages and inside the epidermis. Since these spots are not always present, and since similar spots can be produced by some bacteria, high alkalinity and stress, they are not considered a reliable sign for preliminary diagnosis of this disease. Other signs of WSSV include lethargy, sudden reduction in food consumption, red discoloration of body and appendages and a loose cuticle (Figure 1).

Figure 1: A. Black Tiger Shrimp (*Penaeus monodon*) infected with WSSV, showing characteristic circular white spots on the cuticle of exoskeleton, abdominal segments and carapace. B. Pacific White Leg Shrimp (*Litopenaeus vannamei)* infected with WSSV, showing white spots on abdominal segments and carapace, loosened carapace with white spots (C.), detached carapace with large white spots (D.) and red discolouration of tail appendage and extremities (E.). (*Source: ICAR-CIBA (2016) White Spot Disease of Shrimp*)

The Virus

The virus was initially given a variety of names by researchers located in different countries who perceived the outbreaks to be caused by different viral agents. These early names included hypodermal and hematopoietic necrosis baculovirus (HHNBV), rod-shaped nuclear virus of *Penaeus japonicus* (RV-PJ), systemic ectodermal and mesodermal baculovirus (SEMBV), white spot baculovirus (WSBV), and *P. monodon* non- occluded baculovirus (PMNOB) (Durand et al., 1997; Karunasagar et al., 1997; Sahul-Hameed et al., 1998). All of these agents are currently recognized as one virus, which is called WSSV. It is an enveloped, bacilliform double-stranded DNA-virus (Figure 2). Both the size of the virion (up to 350 nm in length) and the size of the genome (300 kbp) are exceptionally large. The virus was assigned to a newly created family, called Nimaviridae- “nima” means “thread” in Latin (Mayo, 2000), according to the nomenclature assigned by the International Committee on Taxonomy of Viruses (ICTV), and placed in the genus, Whispovirus . WSSV stands alone in this family group and has only distant genomic resemblance to other DNA viruses such as pox, herpes and baculovirus (Mayo, et al. , 2002; Vlak et al., 2005). Based on genetic analyses and morphological features, WSSVs were recently classified as members of White spot syndrome virus, the sole species of genus Whispovirus, the only genus in a monotypic family Nimaviridae (Sanchez - Paz, 2010). WSSV is a large, nonoccluded, enveloped, rod-shaped to elliptical DNA virus with a thread-like tail at one end, sometimes observed in negatively stained electron micrographs and the viruses are found to multiply in the nucleus (Durand et al.1997).

WSSV is a rod-shaped double-stranded DNA virus spanning a length of 240-380 nm and a diameter of 80-120 nm. The virions are composed of a third-layered outer membrane, sometimes with a tail-like extension at one end. The nucleocapsid is contained within the envelope and is a stacked ring structure composed of globular protein subunits of 10 nm in diameter arranged in 14 –15 vertical striations located every 22 nm along the long axis, giving it a cross-hatched appearance as shown in Figure 2 (Leu, et al. 2005 ; Tsai, et al. 2006).

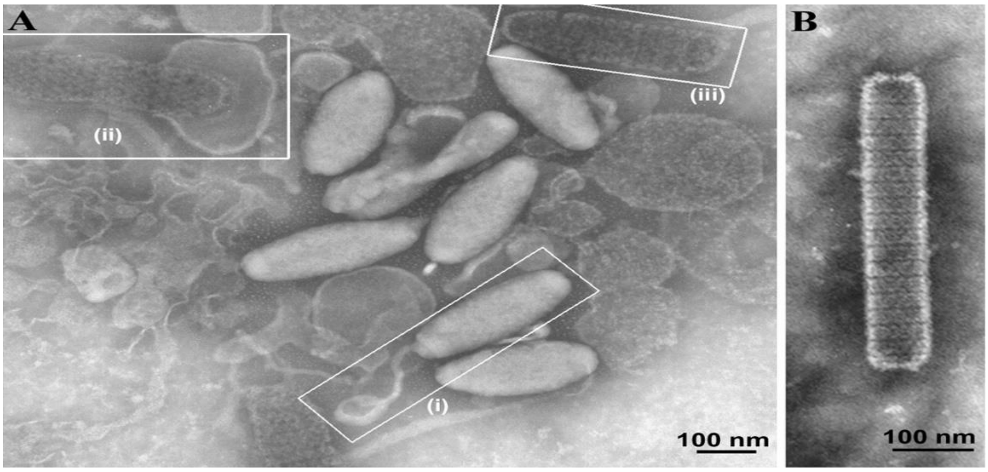


Figure 2 Negative contrast electron micrograph of WSSV viral particle (a) mature WSSV virions with characterized tail-like structure and (b) nucleocapsid (*Source: Leu, et al. 2005*)

Genome Characteristics

The virus genome is a circular, double stranded DNA of around 300 kbp in length. The genomes of three isolated WSSV from China (Yang, et al. 2001), Taiwan (Lo & Kou, 2001) and Thailand (van Hulten et al. 2001) have been completely sequenced. Their genome sizes are 305 307 and 297 kbp, respectively, which demonstrate a slight variation by genetic comparison s. The nucleotide identity between these isolates is 99.3% (Marks et al. 2004). WSSV contains 531 putative open reading frames (ORF) determined by sequence analysis, which could potentially encode functional viral proteins. Almost one third of the predicted ORFs are nonoverlapping and about 80% of them possess the sequence considered as polyadenylation site (AATAAA) downstream of those ORFs. The function of some proteins encoded by predicted ORFs, including structural proteins and non-structural proteins, have been documented

Most of the predicted open reading frames in its ~300 kb genome encode polypeptides that show no homology to known proteins, while the identifiable genes are those coding for structural proteins and some involved in nucleotide metabolism and DNA replication. Nine homologous regions of highly repetitive sequences are found in the genome. Functional studies have identified several WSSV genes, including eighteen immediate early genes, two latency-related genes and four anti-apoptosis genes. WSSV genes are classified as immediate early, early, late or very late genes based on their temporal expression patterns and are regulated as coordinated cascades under the control of different promoters (van Hulten et al. 2001).

The variations associated with the three minisatellites ORF 94, ORF 75 and ORF 125 were carried out for genotyping WSSV and for epidemiological research (Shekhar & Ravichandran, 2007; Pradeep et al. 2008). Studies on the characterization of variable genomic regions worldwide will help explain the evolution of WSSV and its significance to virulence.

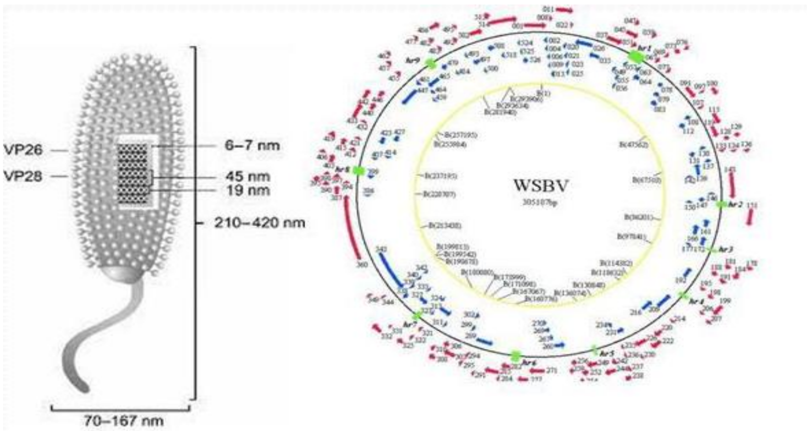
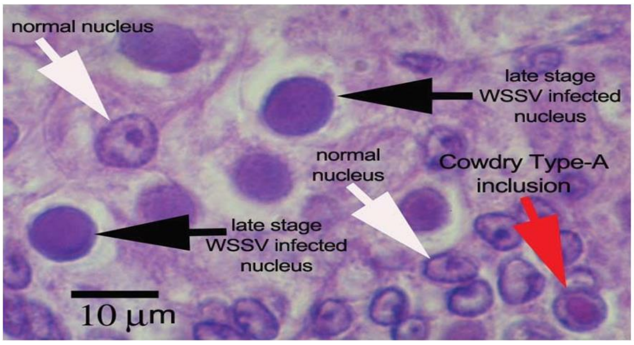


Figure. 3: Structural Features of WSSV and its Genomic Characteristic Details (*Source: Bir, et al. 2017*)

Pathology

The virus causes systemic infections that show characteristic lesions in tissues of ectodermal and mesodermal origin. It does not affect tissues of endodermal origin (e.g. midgut and hepatopancreatic epithelia), though it does infect cells in the interstitial tissue of the organ (mesodermal origin). The virus primarily targets the lymphoid organ, hepatopancreas, gills, and muscle tissues, causing severe pathological changes and ultimately leading to high mortality rates in affected shrimp populations. Quantitative pathogenic analysis have shown that the major target tissues are gills, stomach, cuticular epithelium, hematopoietic tissues, lymphoid organ and antennal glands (Durand & Lightner, 2002; Escobedo-Bonilla et al. 2007).

In early stages of viral development, the hypertrophied nuclei of infected cells show an acidophilic (reddish) central inclusion surrounded by a thin non-stained zone that is framed by a basophilic (blue) ring of marginated chromatin. In the later stages of infection, the central inclusion expands to fill the whole hypertrophied nucleus and it becomes progressively more basophilic with age (Alday & Flegel, 1999), Figure 4 shows the cytopathological changes brought about in WSSV infected tissues when examined microscopically.



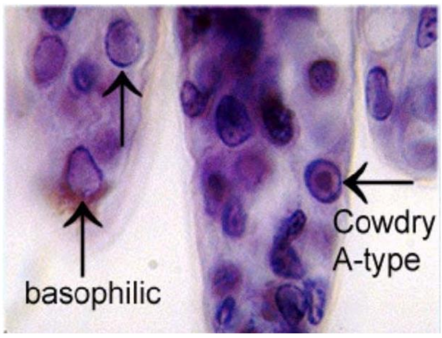


Figure 4.1 Histopathological characteristics of white spot disease: normal nucleus (white arrows); infected cells will have hypertrophied nuclei, which are stained red in the first stage, called Cowdry type A inclusions (red arrow); in the later stages they stain dark blue (black arrows) (H&E stain). Histopathological characteristics of white spot disease in gill tissue after rapid staining: in the early stages the nuclei of infected cells become hypertrophied and stain red; this is called a Cowdry type A inclusion. Figure 4.2 In the later stages the nuclei are basophilic and stain dark blue (*Photos Courtesy of Prof. Timothy W. Flegel*)

Epidemiology and Transmission

The transmission of WSSV occurs through multiple routes. Horizontal transmission of viral particles between shrimp and other Decapod crustacea can occur via 2 routes: (1) Oral, by consumption of tissues from infected hosts including consumption of infected tissue by predation and cannibalism, (2) Via water borne in which the virus invades through gills and other body surfaces by direct exposure to virus particles in water or by cohabitation (Lo et al. 1997; Chou et al. 1998). Vertical transmission occurs via trans-ovum or per ovum from broodstock to offspring (Lo et al., 1997: Sanchez-Paz 2010; Pradeep et al. 2012). and from infected broodstock to progeny (Lo et al., 1996). The transmission of WSSV between different geographical locations can be facilitated by the transport of live and frozen uncooked shrimp (Nunan et al. 1998) and import of broodstock (Stentiford et al. 2012). Environmental factors, such as water temperature, salinity, and the presence of other infectious agents, influence the epidemiology of WSSV outbreaks in shrimp farms (Escobedo- Bonilla et al., 2008).

Diagnosis of WSSV

Rapid and accurate diagnosis is essential for implementing timely control measures and preventing further spread of the virus .There are 3 types of commonly deployed diagnostic methods for the routine detection of WSSV infection, which are discussed below. Electron microscopy remains the gold standard that helps visualize the WSSV particles but is an expensive technique and requires access to an electron microscope facility.

<u>Histopathological Tests</u>

In general, WSSV induced disease is rather easy to diagnose histologically because of the tremendous number and widespread distribution of infected cells present in a moribund penaeid shrimp.

<u>Immunological Tests</u>

These methods are based on monoclonal or polyclonal antibodies produced against viral antigens or recombinant viral antigens. Monoclonal and polyclonal antibodies produced against VP28 or rVP28 have been used to develop several methods including immunofluorescence, immunohistochemistry (Poulos et al., 2001; Anil et al., 2002; Escobedo-Bonilla et al., 2007), immunoblot assays (Anil et al., 2002; Makesh et al. , 2006), immunochromatographic test strips (Sithigorngul et al., 2011), enzyme linked immunosorbent assay (ELISA) (Liu, W. et al., 2002), and western blotting (Yoganandhan et al., 2004). These methods are confirmatory to detect infection and demonstrate infected cells or quantitate viral antigens .However, immunological tests are dependent on the expression of viral antigens and specificity of the antibody against viral antigens.

<u>Molecular Tests</u>

Subsequent to whole genome sequencing of various isolates from different regions of the world, molecular methods, based on polymerase chain reaction (PCR) has been deployed for the detection and confirmation of WSSV infection in shrimp. Later, Nested or two step PCR has been recommended by OIE (Fig 5) which has been recognized to be more than the single step PCR (Lo et al 1998).

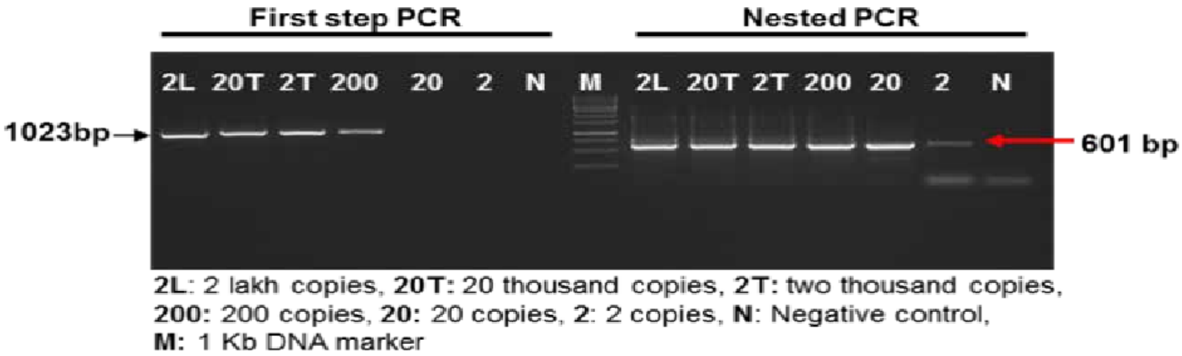


Figure 5. Two Step Nested PCR testing for WSSV infection. Left side gel image shows amplification by first set of primers. Right side gel image shows PCR amplification by second set of primers. (*Source: ICAR CIBA (2016) Molecular Diagnosis of Shrimp Diseases-A Training Manual*)

For getting a quantitative estimation of the virus titer, a real time PCR (RT PCR) has been used (Durand & Lightner 2002). Loop mediated isothermal amplification (LAMP) is another molecular technique which is non quantitative, probe less,highly specific and 10 fold sensitive compared to nested PCR. Also other added advantage is that its rapid and amplification occurs under isothermal conditions, without the need of a sophisticated machine for thermal cycling,making it convenient to use under low resouce settings (Kono et al.2004).

Prevention and Control Strategies

Preventive measures such as strict biosecurity protocols, quarantine procedures, and the use of Specific Pathogen-Free (SPF) broodstock can help minimize the risk of WSSV introduction into shrimp farms (Nunan et al., 2014). Additionally, vaccination, probiotics, and antiviral agents are being explored as potential control strategies to mitigate the impact of WSSV outbreaks (Flegel, 2012).

Several strategies are being deployed for the sustainable management of WSSV. These vary depending on the resources, technical knowhow and facilities available at different locations. They are aimed at different mechanisms and approaches to bring about an effective control (World Bank, 2014). Some of these strategies have been listed below:

1. Exclusion of WSSV vectors and carrier organisms (sludge removal, liming, complete dry out between culture cycles, proper fencing, etc.)
2. Greenhouse shrimp production (cultivation under environmental controlled conditions to prevent the negative stress impacts)
3. Disinfection and batch production (stocking ponds should be disinfected before starting a new production cycle).
4. Immune stimulation (inclusion of prebiotics, probiotics and herbal immunostimulants in nutrition.
5. Vaccination against WSSV (monovalent or multivalent vaccines using envelope proteins like V28, V19, V26 and DNA vaccines.
6. Gene Silencing (RNAi triggered by the application of short ds RNA duplexes), mi-RNA have been tried to inhibit WSSV.
7. Gene Editing Technologies like CRISPR-Cas for generating viral resistant stocks are being tried
8. Quarantine measures Biosecurity and quality testing of export import shrimp stocks should be strictly followed.
9. Specific Pathogen Free (SPF- production of pathogen free stocks in bio secure and quarantined facility and pathogen testing by PCR, etc.
10. Specific Pathogen Resistance (SPR-production of shrimps resistant to single or multiple pathogens by the breeding program.
10. Stocking Density (Maintenance of low stocking densities in shrimp culture reduces stress that helps decrease disease incidence.

Future Perspectives

Advancements in genetics, breeding molecular biology, genomics, bioinformatics and immunology offer promising opportunities and avenues for developing novel diagnostic tools and therapeutic interventions against WSSV.

Furthermore, sustainable aquaculture practices, coupled with effective disease management strategies, are essential for ensuring the long-term viability of the shrimp farming industry in the face of WSSV and other emerging pathogens.

Conclusion

WSSV remains a significant threat to global shrimp aquaculture, highlighting the need for continued research efforts aimed at understanding the virus's biology, epidemiology, and pathogenesis.

By adopting integrated disease management approaches and implementing stringent biosecurity measures, stakeholders can effectively mitigate the impact of WSSV outbreaks and promote the sustainable growth of the shrimp farming industry.

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