

A critical review on White Spot Syndrome Virus (WSSV): A potential threat to shrimp farming in Bangladesh and some Asian countries

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Abstract

WSSV is one of the alarming pathogen all over the world especially for the tropical countries. It causes huge economic loss through rapid mortality of crustacean and some of important mollusks species. Immediately after occurrence in 1992, this disease continuously depletes the world aquaculture production. It is more severe in Asian country and high prevalence in winter sometime after heavy rainfall. Morphologically WSSV consists with a double strand DNA with a 6-7 nm thick envelope, a nucleocapsid and proteins. The nested PCR is the most reliable technique to detect WSSV DNA from shrimp products. Farmer should be given additional concern to prevent the outbreak this virulence virus. Therefore the current study aim is to focus on some biological and economical aspects of WSSV pathogen. This will be an useful reviews article for the aquaculture science.

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Introduction

Status of shrimp farming in world

Shrimp aquaculture is foremost earning industries in many tropical and subtropical countries of the world especially in Asia and Australia. It has a very significance contribution in national income, food security, employment opportunities to both rural and coastal people and eventually in poverty alleviation. According to Food and Agriculture Organization (FAO, 2014), Shrimp is occupying the largest single commodity in international market and contribute about 15 percent of the total value of traded fishery products in 2012.

The FAO and GOAL survey estimate that the global production of farm-raised shrimp will reach 3.8 million tonnes in 2012 (Fig. 1) and 4 million tonnes in 2013 (Valderrama *et al.*, 2012). About 50 percent shrimp are captured from the Northwest and Western Central Pacific ocean, Indian Ocean and Western Atlantic Ocean also contribute 20 and 17 percent of the total respectively. In the world of shrimp aquaculture, the most important two species are the *Penaeus vannamei* and *Penaeus monodon*, sometime *Penaeus indicus* also attain important for farming. In Asia most shrimp aquaculture occurs in China, followed by Thailand, Indonesia, Vietnam, India, and Bangladesh (Fig. 2).

Therefore In Bangladesh, shrimp farming has rapidly expanded since 1980s (Debnath *et al.*, 2014) and now this sector is the second largest export earnings sources contributing about 5% to national GDP (Hossain *et al.*, 2014; Rahman and Hossain, 2013) and approximately 8.5 million Bangladeshi peoples particularly coastal regions peoples directly depend on this sector for their livelihood (DoF, 2013). The main cultured species is black tiger shrimp (*Penaeus monodon*), and in 2010-2011 Bangladesh produced 56,569 MT of tiger shrimp with an export value of approximately \$462 million (DOF, 2012).

The major viruses of concern for shrimp aquaculture are white spot syndrome virus (WSSV), yellow head virus (YHV), taura syndrome virus (TSV), infectious hypodermal and haematopoietic necrosis virus (IHHNV), infectious myonecrosis virus (IMNV), gill-associated virus (GAV), monodon slow growth virus (MSGV) and monodon baculovirus (MBV) (Walker and Mohan, 2009).

In recent year's White Spot Syndrome Virus (WSSV) is a major threat to Shrimp Aquaculture in many country especially Asian countries. As a result shrimp production was drastically decreases and many shrimp farmer and hatchery owner had to face huge economic loses. But they have no clear idea about the syndrome of this WSSV virus. So the objective of this review article is to give clear idea about some biological aspect of WSSV virus and the consequence of WSSV virus with some mitigation procedure and economic aspects.

White Spot Syndrome Virus (WSSV)

White spot syndrome virus (WSSV) is a pathogen responsible for the white spot diseases (WSD) in cultured penaeid shrimp. WSSV is the only member of the genus Whispovirus, and family, Nimaviridae (Reddy *et al.*, 2013; (Walker and Mohan, 2009). Depending on clinical sign initially WSSV virus was known in different names like Hypodermal and haematopoietic necrosis baculovirus (HHNBV), chinese baculovirus (CBV), systemic ectodermal and mesodermal baculovirus (SEMBV), penaeid rodshaped DNA virus (PRDV) or rodshaped nuclear virus of *Penaeus japonicus* (RV-PJ), and white spot disease (WSD) to the researcher (Reddy *et al.*, 2013)

History of outbreak and world Distribution

White spot disease (WSD) was first reported in June 1992 in cultured kuruma shrimp (*Penaeus japonicus* Bate, 1888) in the Fujian Province of China and in nearby Taiwan (Jiang 2009; Walker and Mohan, 2009). The disease then spread to Japan in 1993 where it was reported from farmed *M. japonicus* (Walker and Mohan, 2009; Nakano *et al.*, 1994).

Over the next few years the disease became widespread throughout Southeast Asia, spreading to Vietnam, Thailand, Malaysia, Indonesia, and India, causing hundreds of million dollars economic losses for the shrimp industry every year. In Bangladesh, WSSV was first identified in 1994 from a semi-intensive farm in Cox's Bazar, and then subsequently in 1996 the disease spread to Khulna region and other southwest part country, affecting approximately 90% of extensive shrimp farms and causing a 20% drop in national shrimp production. As a result the shrimp exports in Bangladesh dropped from 25,742 tonnes to 18,630 tonnes in 1997–1998 (Debnath *et al.*, 2014). The first recorded outbreak of WSSV in the Americas was at a farm in Texas in November 1995 (Lightner *et al.*, 1997). In 1999 WSSV first appeared in Panama and within two months the disease spread north to Honduras and Guatemala.

In late 1999, WSSV spread in Ecuador (Chakraborty and Ghosh, 2014) therefore drastically down their shrimp export (nearly 70%) (Walker and Mohan, 2009). It was anticipated that the reasons behind this rapid spread are mainly due to the potency of the virus, lack of its awareness and prevention, global expansion of the industry and increasing intensive shrimp farming practices (Chakraborty and Ghosh, 2014). WSSV also reached Spain and Australia in 2000–2001. In both cases, successful containment and eradication were reported and for both events (OIE, 2013)

WSSV infects a wide range of aquatic crustaceans ranging from marine, brackish water and freshwater captured and cultured crustaceans and other arthropods. It has found positive WSSV in PCR test for about 18 penaeid shrimp, eight caridean species, seven species of lobster, seven species of crayfish, 38 crab species, and six non decapod crustacean species (Chakraborty and Ghosh, 2014). The most affected species are penaeid shrimps (*P. indicus*, *P. japonicas*, *P.*

merguiensis, *P. monodon*, *P. penicillatus*, and *P. vannamei*); other shrimp (*Acetes* sp., *Exopalaemon orientalis*, *Macrobrachium idella*, *M. lamerrae*, *M. rosenbergii*, *Metapenaeus dobsoni*, *M. ensis*, *Palaemon adspersus*, *P. sirrifer*, *P. styliferus*, *Parapenaeopsis stylifera*, *Scyllarus arctus*, *Solenocera indica*, *Squilla mantis*, *Trachypenaeus curvirostris*); crabs (*Calappa lophos*, *Portunus sanguinolentus*, *Charybdis* sp., *Helice tridens*, *Paratelphusa hydrodomous*, *P. pulvinata*); wild lobster (*Panulirus* spp.); copepods; pupae of Ephyridian insects; crayfish (*Orconectes punctimanus* and *Procambrus clarkii*); pest crab (*Sesama pictum*); mud crab (*Scylla serrata*); and many other marine crustaceans (Reddy *et al.*, 2013; Rajendran *et al.*, 2001; Flegel, 2006).

Morphology and Structure of WSSV

WSSV is a double-stranded DNA virus, measuring 80–120nm in diameter and 250–380nm in length (Fig. 3 A) (Durand *et al.*, 1997). The virions are rod-shaped to elliptical in form, and have a unique flagella-like appendage at one end (Walker and Mohan, 2009). Virions comprise at least 45 structural proteins that are arranged in three morphologically distinct layers (Tsai *et al.*, 2004; Li *et al.*, 2007). The virions replicate inside the nuclei of infected cells without the production of occlusion bodies. WSSV targets tissues are ectodermal (cuticular epidermis, foregut and hindgut, gills and nervous tissues), and mesodermal (connective tissue lymphoid organ, antennal gland and hemopoietic tissue) (Wongteerasupaya *et al.*, 1995).

The nucleocapsid proteins include a basic DNA-binding protein (VP15) and a giant protein (VP664), which forms the stacked ring subunits (Leu *et al.*, 2005; Witteveldt *et al.*, 2005). The viral envelope is 6–7 nm thick and has the structure of membrane with 35 different proteins of which VP28 and VP26 are the most abundant, accounting for approximately 60% of the envelope and (Sánchez, 2010; Walker and Mohan, 2009).

Genomics study of WSSV virus

The genome of WSSV is like circular dsDNA molecule (Fig. 3 B) and is one of the largest animal virus genomes that have been entirely sequenced (Hulten *et al.*, 2001). The WSSV genome can be divided into a) structural genes which encode for envelope and nucleocapsid or integument, b) functional genes involved in the virus proliferation and life cycle function, c) the latency related genes whose expression can be detected even though the structural genes might not be active and d) temporal regulatory genes which participate at specific times during the infection. The genome size varies according to the viral isolate.

Three complete WSSV sequences (accession numbers AF369029, AF332093, AF440570) identified and the sizes of the genomes were found to be 292,967bp, 305,107bp, 307,287bp for the Thailand, China and Taiwan isolates, respectively (Sánchez 2010, Kang *et al.*, 2009). The nucleotide sequence analysis revealed that the WSSV genome encodes approximately 185 open reading frames (ORFs) of 50 amino acids or more.

Clinical Syndromes and Pathology of WSSV

WSSV is very virulence pathogen and considered as the most dangerous and devastating disease in shrimp aquaculture. Though it takes some time to express but once after its expression, infected animals stock die within 3-8 days resulting in high mortality (Hossain *et al.*, 2014).

The WSSV infected shrimp in the field is found to gather near the pond edge and display clinical signs in 1 or 2 days before occurrence of any mortality. Cumulative mortality may reach 100% within 10 days after the onset of disease (Hossain *et al.*, 2014). In grow-out ponds, juvenile shrimp of all age and sizes are susceptible to the disease but massive mortality occurs 1 or 2 months after stocking (Kasornchandra *et al.*, 1998). The gross pathological signs are loss of carapace, dark reddish or pink coloration on body surface,

presence of circular white spots or patches of 0.5-3.0 mm in diameter most prominent in the cuticle of cephalothorax and tail (Reddy *et al.*, 2013; Wang *et al.*, 2002; Rout *et al.*, 2005). Other symptoms of the disease include fewer intakes of feed, reduced preening and low response to stimulus and loose cuticle (Reddy *et al.*, 2013).

Histologically, infection is characterized by eosinophilic to progressively more basophilic inclusion bodies in the hypertrophied nuclei of infected cells (Lo *et al.*, 1996). Infected nuclei become progressively more basophilic and enlarged (Hameed *et al.*, 2003). Karyorrhexis, cellular disintegration and vacuolization may occur in the last stage of infection (Kasornchandra *et al.*, 1998; Reddy *et al.*, 2013).

Detection of WSSV from Shrimp tissue

A number of diagnostic procedures have been developed for detection of WSSV. These include gross observation, histopathological techniques (Reyes *et al.*, 2009) in situ hybridization (Lightner and Redman 1998), immunological methods such as Nitrocellulose-enzyme immunoblot (Nadala and Loh 2000) and Western blot techniques (Durand *et al.*, 1997) and more recently highly simple, sensitive and reliable technique such as Polymerase Chain Reaction based methods (Kim *et al.*, 1998). The sensitivity of these techniques may vary depending on DNA based.

Preliminary WSSV can be detected by observing external appendages. Severely infected shrimp often have a loose cutical with white spots of 0.5 - 2.0mm in diameter which are most apparent on the inside surface of the carapace (Hossain *et al.*, 2014). This white spot due to abnormal deposition of calcium salts by the cuticular epidermis. In many cases shrimp displayed a pink to reddish-brown colouration due to expansion of the cuticular chromatophores.

For histopathological detection white spot syndrome virus infected tissue are stained by

Hematoxylin and eosin therefore presents eosinophilic inclusion (Fig. 4) bodies with hypertrophied (swollen) nuclei and marginated, slightly basophilic, chromatin. The nuclear hypertrophy is due to the development and accumulation of developing virions within the

nucleus. The inclusions become more basophilic in appearance in later stages of development, (Durand *et al.*, 1996, 1997; Lightner, 1996). The virus severely damages the stomach, gills, subcuticular epithelial cells, lymphoid organ, antennal gland and haemocyte.

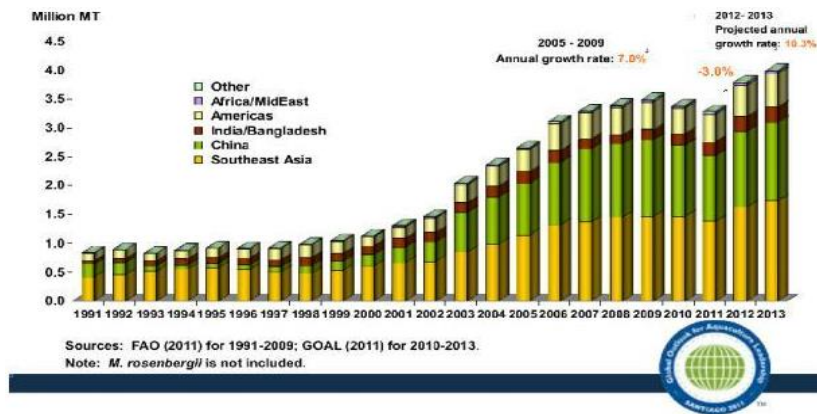


Fig. 1. Global shrimp production trend during 1990-2013.

Source: FAO (2014) for 1991-2009, GOAL (2011) for 2010-2013.

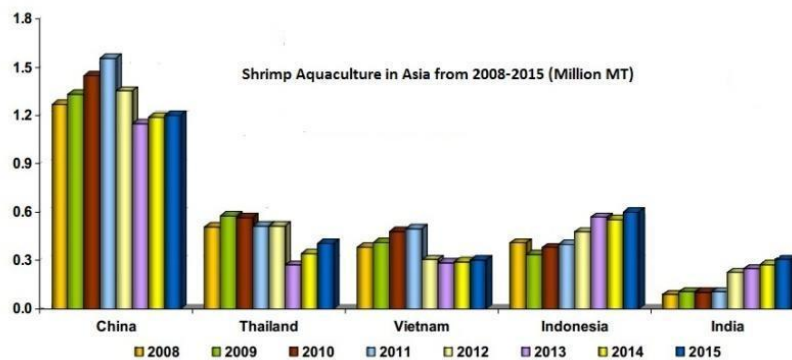


Fig. 2. Shrimp production in Asia during 2008-2015.

Source: Undercurrent News, E1 Studios, Unit 107, 7 Whitechapel road, London E1 1DU, UK, and GOAL 2010-2013).

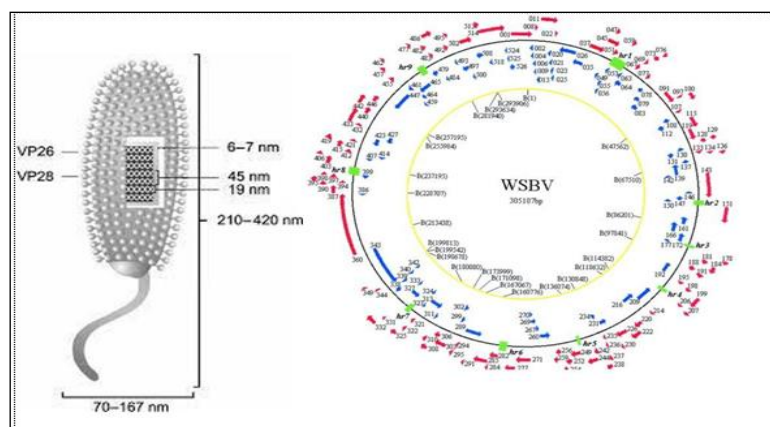


Fig. 3. A. Shows the general structure of WSSV; **B.** Showing the genome pattern.

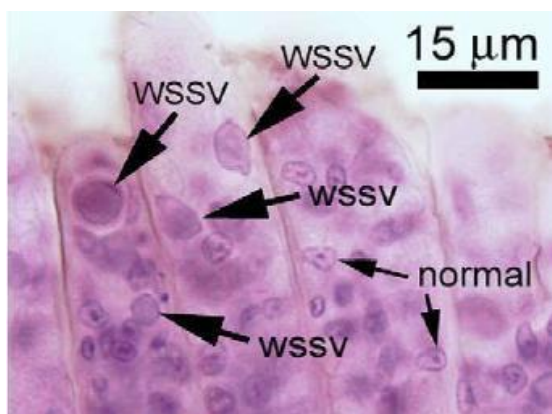


Fig. 4. Hematoxylin/Eosin section of WSSV infected gill tissue of shrimp. The arrow indicates the presence of virus. (Flegel, 2006).

Among the various diagnostic techniques, PCR provides a high degree of sensitivity and specificity in detection of WSSV (Hossain *et al.*, 2004). PCR has been used recently to detect WSSV in a very specific and sensitive manner. Nested or two step PCR has the advantage of increasing the level of sensitivity over single step PCR. When a shrimp shows clinical signs of WSSV, it is easily detected by single step PCR.

At present, nested PCR method is recognised to be the most effective diagnostic tool for this pathogen. For the development of effective diagnostic tools, number of primer and also WSSV genomic library has been constructed and analysed. Although the PCR techniques have proved themselves as highly sensitive for detection of WSSV, there are still limitations for their widespread application such as requirement of special equipment, expensive reagents and well trained personnel (Walker and Mohan 2009)

Various environmental factor especially temperature strongly influence the expression of the WSSV virus. The most WSSV occurrences in winter season at low temperature. According to Vidal *et al.* (2001) at temperature above 32°C WSD diseases did not spread to the *L. vannamei* shrimp, but at 26°C WSD quickly developed with 100% mortality of the same species.

Temperature fluctuation greater than $\pm 3^{\circ}\text{C}$ are the conducive to the outbreak of WSSV (Esparza *et al.*, 2010). So the information always provided to farmers manages WSSV by avoiding stock in cool season or controlling temperature year round by greenhouse. Heavy rainfall events may also precipitate the outbreaks of WSSV due to the combined effect of a rapid drop in both salinity and temperature (Tendencia *et al.*, 2011). This diseases appears to be due to the cumulative effect of a reduced host immune response and an increase in the rate of viral replication at temperatures less than 28°C (Moser *et al.*, 2012).

Economics consequences of WSSV Virus.

WSSV causes huge economic loss in world aquaculture (Table 1). After the first outbreak in 1992, it reduced the production over 70% resulting in a production loss of over US\$ 2 billion in China in three years. In Thailand, before the hit of WSSV, annual production rate was roughly 34,000 tonnes per year, but in 1994 the their production reduced to 2,65,000 tonnes, or US\$ 1.6 billion by value. Indonesia also exhibited a similar trend, as production until the WSSV outbreak was steadily increasing at a rate of 17,000 tonnes per year from 1985 until 1991.

In 1992 due to the WSSV their production dropped resulting potential production losses of roughly US\$ 1 billion through ten years. In Ecuador, outbreak occurred at 1999 and from that time production decreased over 60% in two years, resulting in losses of over US\$ 1 billion from 1998-2001 (Chakraborty and Ghosh, 2014). The same situation also reported in Panama, and Peru production dropped by 90% resulting in losses of over US\$ 100 million and US\$ 70 million respectively over three years.

In India since 1992, shrimp aquaculture, India's earnings have gone down to a loss amount of INR 2700 million (Mishra 2012). In 1994 the disease spread to southwest region of Bangladesh, affecting approximately 90% of extensive shrimp

farms and causing a 20% drop in national shrimp production. Therefore, Shrimp exports fell from 25,742 tonnes to 18,630 tonnes in 1997–1998 (Debnath *et al.*, 2014)

Table 1. Economic loss of Shrimp production due to diseases. *Modified from Israngkura et al.*, 2002).

Country (Year)	Estimated Loss (mt)	Loss as Percentage of Expected Outputs (%)	Value of Production Loss (US\$ Million, 1994)
Thailand (1994)	130	58	650
Philippines (1989)	57	96	285
Ecuador (1992)	34	27	170
Indonesia (1991)	50	34	250
China (1992)	180	84	900
Taiwan (1987)	100	72	500
Mexico (1994)	1	8	5
USA (1993)	12	NA	60
India (1994)	25	36	125
Vietnam (1994)	10	20	50
Bangladesh (1994)	5	14	25
Total	541	74	3,019

Conclusion

WSSV is the most virulence emergent diseases that cause rapid mortality of crustacean like shrimp, prawn and crab consequently causes huge economic loss in global aquaculture. This diseases also greatly impact on human and ecosystem health. The WSSV DNA is not destroyed by icing, freezing, and cooking followed by slow freezing, canning, and cold storage processes. So, better management practices are the only way to get remediation from this hazard. Attention should be given on all stages of the production cycle from hatchery operation to pond bottom preparation and water management prior to stocking, seed selection and stocking, and post-stocking management of ponds. It is the high time to conduct effective research for the development of detection methods and ecological studies of WSSV in wild populations should be a prime priority. The good news, that farmer are

now more concern on WSSV and strictly follow the regulation of good aquaculture practice (GAP) like water monitoring, uses of PCR treated fry and other accessories management, therefore the intensity of this diseases is now under control.

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