A review on White Spot Disease in penaeid shrimp farms with a brief study on its occurrence in shrimp aquaculture zones.

Code of Practice in Iran.

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Introduction

- At the moment, White Spot Disease is playing a dominant role in devastating the shrimp farming all over the world.

- The first outbreak due to WSSV was reported in shrimp farms in Taiwan in 1992, followed by other shrimp farming countries of South East Asia, Middle East, North, Central and South America.

- The WSD can be prevented and its impacts can be mitigated through implementing scientific health management strategies, and application of principles of biosecurity at the pond, farm, national and regional levels.

- Knowledge about the nature of the pathogen, its carriers and routes of entry are very important.

- Biosecurity principles primarily target pathogen exclusion from the culture environment and host.

- Biosecurity principles can be best applied to pathogens that are well studied.
Nature of the WSD

- White spot disease (WSD) is a highly contagious viral disease of penaeid shrimp (family Penaeidae), characterized by the rapid onset of high levels of mortality in farmed shrimp populations.

- Outbreaks are preceded by cessation of shrimp feeding followed within a few days by the appearance of moribund shrimp at the edge of ponds and then mass mortality.

- WSD has exhibited panzootic behavior in Asia and the Americas.
Aetiology

- The causative agent of White Spot Disease is White Spot Syndrome Virus (WSSV) which is an enveloped, double stranded DNA virus.

- In the 8th report of the International Committee on Taxonomy of Viruses (ICTV) in 2004, WSSV was assigned as the only member of the genus *Whispovirus* within the *Nimaviridae* family. Virions of WSSV are ovoid or ellipsoid to Bacilliform in shape, have a regular symmetry, and measure 120–150 nm in diameter and 270–290 nm in length. Most notable is the thread- or flagella-like extension (appendage) at one end of the virion.

![WSSV particles in longitudinal and cross section in nucleus, *L.vannamei*](image)
Susceptible species

- All decapod crustaceans (order Decapoda), including shrimps, lobsters and crabs from marine, brackish or freshwater environments, are considered susceptible to infection.
World Distribution

Prior to 1992, global shrimp production had been increasing consistently year after year since data were first recorded in 1970. For the first time, in that year, a disease known as White Spot Syndrome began to cause devastating fatalities in the T’aipei Taiwanese province shrimp farms and subsequently in the Fuzhon and Quangzhou provinces in China.

By 1994 the syndrome had spread by imported shrimp to southern Japan, through Thailand, into Indonesia and as far west as the coast of India.

A year later, the disease leapt over the Pacific Ocean and spent the following three years spreading throughout small scale farms in North America. Not until late 1998 or 1999 did the disease begin to ravage South America, first in Ecuador, then Peru and the rest of the Pacific shrimp farming countries.

In 2002, the WSD was seen in the Middle East in Iran.

In less than ten years this disease appeared and spread to global extent, creating by far the greatest economic damage of any of the other diseases like TSD and YHD.

The reasons for this rapid spread are combination of the strength of the disease, lack of awareness and prevention, internationalization of the industry and increasingly intensive farming practices.
### Occurrences in Northern Shores of Persian Gulf & Oman Sea

- **Occurrence of WSD sorted by farmed species in Iran**

<table>
<thead>
<tr>
<th>WSD Suffered</th>
<th>Farmed shrimp species</th>
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<tbody>
<tr>
<td>Provinces</td>
<td>P. indicus</td>
</tr>
<tr>
<td>Boushehr</td>
<td>2005</td>
</tr>
<tr>
<td>Sistan va Baluchestan</td>
<td>2008</td>
</tr>
<tr>
<td>Hormozgan</td>
<td>No report</td>
</tr>
<tr>
<td>report</td>
<td></td>
</tr>
</tbody>
</table>

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![Map of Iran showing Persian Gulf and Oman Sea](map.png)

- **Khuzestan**
- **Boushehr**
- **Sistan va Baluchestan**
- **Hormozgan**
- **Persian Gulf**
- **Oman Sea**
- **Caspian Sea**
- **Persian Gulf**
- **Oman Sea**
Epizooology

It this section the followings will be discussed;

- Disease pattern,
- Transmission,
- Prevalence and mortality,
- Environmental stressors,
- Vectors,
- Immunostimulants & vaccine.
Disease pattern

- WSSV Infection sometimes causes disease and sometimes not, depending on factors as yet poorly understood but related to species tolerance and environmental triggers.

- Persistent infection occurs commonly and lifelong infection has been reported.

- Co-infections of different viruses including Hepatopancreatic Parvovirus (HPV), *Penaeus monodon* Baculovirus (MBV) and IHHNV together with WSSV have been reported.
Transmission of WSSV

- The infection can be transmitted:
  - vertically (trans-ovum),
  - horizontally by consumption of infected tissue (e.g. cannibalism, predation, etc.), and
  - by water-borne routes

- Birds such as gulls may mechanically transmit infection between ponds by releasing captured, moribund or dead shrimp.

- Also the role of fomites such as farm equipments and people should not be neglected in mechanically transmission of the virus particles in the heavy presence of virus in environment.
Prevalence and mortality rate

- The prevalence of WSD is highly variable, from <1% in infected wild populations to up to 100% in captive populations.
- 70-100% mortality in within 3 days after onset of the clinical signs (in *P. monodon* and *P. japonicus*).
- Comparing the reports of mortality rate and virulence of WSSV in Iran in *P. indicus* as occurred in 2004 and 2006, with *L. vannamei* in 2008, the time to reach peak mortality of 100% in *L. vannamei* was longer (7-30 days) than the *P. indicus* (3-7 days).
- Natural outbreaks of WSSV are categorized into per acute, acute to sub acute and chronic forms, where mortality occurs within 2-3 days, 7-10 days and 15-28 days, respectively. In this regard, the outbreak of WSD in *P. indicus* as occurred in 2004 and 2006 was the acute form and the outbreak of WSD in 2008 in *L. vannamei* was the chronic form.
Environmental stressors

- Disease outbreaks may be induced by stressors, as triggers for the expression of the clinical disease, such as:
  - rapid changes in salinity that may act through osmotic stress.

- The outbreak of the WSD in Iran in 2008 (Sistan va Baluchestan Province) had occurred within a week after a heavy raining which made a rapid change in salinity.

- Water temperature has a profound effect on disease expression, with average water temperatures of below ~30°C being conducive to WSD outbreaks.

- Increasing the density of the shrimp per pond also can act as a stressor if the pond environmental factors are poorly managed. In most case of WSD outbreak in Iran, poor management in the farms is believed to be the main stressor for occurrence of the disease.
Vectors

- Vectors of WSD include:
  - rotifers,
  - marine molluscs,
  - polychaete worms, and
  - non-decapodal crustaceans including *Artemia salina* and
  - the copepods, as well as non-crustacean aquatic arthropods such as sea Slaters (Isopoda) and Euphydradae insect larvae.
Immunostimulants & vaccine

- The crustacean innate immune system recognizes molecular patterns shared by large groups of pathogens, such as beta-glucans from fungi and lipopolysaccharides and peptidoglycans from bacteria.
- Also reports have shown that beta-glucan, Vitamin C, seaweed extracts (fucoidan) and other immunostimulants may improve resistance to WSD.

- Several studies have shown that resistance of prawns to WSV can be enhanced by exposure to these compounds. As their efficacy and methods of administration become better defined, immunostimulants may be used to improve the resistance of farmed crustaceans to WSV and other pathogens in an attempt to reduce the risk of disease outbreaks. However, any benefit they may confer is likely to be minimal in adverse environments or in the absence of appropriate disease prevention strategies.

- Although recent studies of crustacean immunology suggest some capacity for acquired immunity, but currently no consistently effective vaccination methods have been developed.
Diagnostic Criteria

- Clinical & gross signs
- Histopathological findings
- Laboratory tests
Clinical & gross signs

- Infected shrimp display clinical signs such as:

  - **White spots** embedded within the which is *not a pathognomic* sign of WSD exoskeleton,
  - **anorexia** displayed as empty intestine and abdomen due to cession of feeding,
  - **lethargy**,
  - **swollen branchiostegites** due to fluid accumulation,
  - **dirty gills**,
  - **separated loose cuticle** from underlying epidermis,
  - **yellowish-white and enlarged hepatopancreas**,
  - **Reddish discoloration** of the moribund shrimp and their gathering around the edge of the pond during the day. A high mortality will happen after 3-7 days up to 100%.
Clinical & Gross sign reported from Iran

- Gross signs in WSD out break in 2008 in *L. vannamei* farms in Iran (Khuzestan province) are reported as lethargic behavior in affected animal, cessation of feeding, followed within a few days by the appearance of moribund shrimp swimming near the surface at the edge of pond with Pink to reddish-brown discoloration of the body and white spot of about 0.5-2 mm on the cuticle, especially on the inner surface of the exoskeleton of cephalothorax and abdomen.

- The cuticle could be easily separated from the underlying epidermis (Fig.8) and the hepatopancreas had become yellowish-white with a swollen and fragile texture.

- Cuticular deformities such as broken or withered antennae and damage rostrum, *opaque abdominal musculature* and melanised gill were consistently observed.

- There was 70-100% mortality in white spot disease affected farms within 7-30 days after the onset of the clinical signs in *L. vannamei* in Iran but the mortality rate in *P. indicus* farms reported from Iran in 2004 and 2006, was reached to 100% within 3-7 days after onset of more or less the similar clinical signs (Afsharnasab M. et al 2009).
Mortality
Moribund Shrimp with Red Discoloration
White spots
Opaque muscle
The histopathology of WSD in moribund shrimps collected during outbreaks is distinctive and can be used for preliminary confirmation of an initial diagnosis. However, additional tests such as PCR, in situ DNA hybridisation, Bioassy, transmission electron microscopy are required for final confirmation (OIE manual of aquatic 2009).

The major targets of WSSV infection are tissues of ectodermal and mesodermal embryonic origin, especially the cuticular epithelium and subcuticular connective tissues.

Although WSSV infects the underlying connective tissue in the shrimp hepatopancreas and midgut, the hepatopancreatic tubular epithelial cells of these two organs are of endodermal origin, and they do not become infected.

Moribund Shrimps with WSD, have systemic viral infection leading to necrosis of tissues of ectodermal and mesodermal origin.

Histological signs of WSSV infection include enlarged nuclei in tissues of ectodermal and mesodermal origin and the most convenient tissue for diagnosis is the subcuticular epithelium. Infection and necrosis are seen in cuticular epithelial cells and connective tissue cells of the stomach, carapace and gills.

Infection is also seen in the antennal gland epithelium, lymphoid organ sheath cells, haematopoietic tissues, and in fixed phagocytes of the heart.

Infected cells typically have hypertrophied (enlarged) nuclei containing a single intranuclear inclusion that are initially eosinophilic and (as an artifact of fixation in Davidson’s fixative solution) are separated by a clear halo from the marginated chromatin. These are known as Cowdry type A inclusions;

They are intranuclear, eosinophilic, amorphous and surrounded by a clear halo beneath the nuclear membrane but later, inclusions become lightly to deeply basophilic and fill the entire nucleus (Lightner D.V. 1996,OIE Manual of Aquatic 2009).

In the histopathological findings in WSD out break in *L. vannamei*, in 2008 (Iran-Khuzestan province) reported by Afsharnasab M. et al. (2009), the subcuticular epithelium of stomach provides excellent view of Cowdry type A inclusion bodies characterized by marginated chromatin separated from nucleoplasm. (Fig. 9). In the out break of WSD in *P. indicus* farms in 2005 (Iran-Boushehr province) reported by Afsharnassab M. et al. (2006) same histological finding were seen in the moribund shrimp.

Above. *L. vannamei*. Iran. The cuticular epithelium is separated from the connective tissue and large basophilic inclusion bodies (arrows) are centronuclear and segregated from the membrane. With the progress of infection the inclusion bodies were separated by a halo 'from the marginal chromatin .(H&E 100X)

Source: M. Afsharnassab
Histopathological findings

Cowdry type A inclusion bodies are intranuclear, eosinophilic, amorphous and surrounded by a clear halo beneath the nuclear membrane but later, inclusions become lightly to deeply basophilic and fill the entire nucleus (Lightner D.V. 1996, OIE Manual of Aquatic 2009).

Above. Intranuclear IBs characteristic of WSSV infection (arrow) in the gill tissue cells of *L.vannamei* in Iran showing signs of WSSV (H&E 100X). Source: M.Afsharnasab.

Left. Intranuclear IBs characteristic of WSSV infection (arrow) in the heart tissue cells of *L.vannamei* in Iran showing signs of WSSV (H&E 100X). Source: M.Afsharnasab.

Histopathological findings

Sections of various tissues from a WSD-infected juvenile white shrimp reacted by in situ hybridisation with a DIG-labelled DNA probe to the virus. The probe has reacted strongly with intranuclear inclusion bodies containing WSD in the various tissues of this shrimp, including the cuticular epithelium of the stomach (Fig 3, 900x), the cuticular epithelium and connective tissues of the carapace (Fig 4, 900x), and epithelial cells in the antennal gland (Fig 5, 450x)

Source: DV Lightner

Histological sections (900x) of the stomachs of blue shrimp (*P. stylirostris*, Fig 1) and white shrimp (*P. vannamei*, Fig 2) experimentally infected with WSD. Both species display severe infections by WSD, with classic WSD intranuclear inclusion bodies (arrows)

Source: DV Lightner
Laboratory tests & procedures

- Laboratory procedures should comply with the *Manual of Diagnostic Tests for Aquatic Animals* (the OIE Aquatic Manual 2009). The recommended minimum numbers of specimens to collect for diagnosis are 100 for the larval stages of most crustaceans; 50 for the postlarval stages; and 10 for juveniles and adults, with preference for individuals with signs and/or gross lesions.

- There are two situations in which WSSV infection requires detection: For confirmation of suspect clinical WSD and In Targeted surveillance (screening) to establish the infection status of asymptomatic populations.

  - **Confirmation of suspect clinical WSD**
    - For confirmation of a suspected outbreak, animals that are representative of those showing clinical and/or gross signs should be sampled. Whole animals, haemolymph, gills, stomach, abdominal muscles and pleopods provide suitable specimens for examination. Although dead animals can sometimes provide useful diagnostic information, they are often unsuitable for examination because of the rapid onset of postmortem changes.

  - There is a higher probability of detecting the virus in crabs than in shrimp. The best life stages of crustaceans for detection are late PL stages, juveniles and adults. Probability of detection can be increased by exposure to stressful conditions (e.g. eye-stalk ablation, spawning, moulting, changes in salinity, temperature or pH, and during plankton blooms)(OIE Manual of Aquatic 2009).

  - **Targeted Surveillance**
    - For keeping apparently healthy populations under surveillance, the number of animals to be tested will depend on the required level of confidence in the findings. Whole larvae, postlarvae and juvenile animals, as well as haemolymph, gills or pleopods from juveniles to broodstock, provide suitable specimens for examination.

    - Two-Step PCR is the preferred test and will be follow-up bioassay to confirm the presence of viable virus in PCR-positive samples if required.

    - Two-step PCR and sequencing are the recommended methods as well for declaring freedom of a country/zone/compartment, only for juveniles and adults and possibly PLs. For such purpose, Two-step PCR negative results are required. Where a two-step PCR positive result cannot be confirmed by sequencing, this also counts as a negative result.(OIE Manual of Aquatic 2009).

    - In non-destructive screening by PCR, it is recommended by OIE aquatic manual to submit (a small piece of) gill, (a small aliquot of) haemolymph or (a small piece of) pleopod. There is also some evidence to suggest that an ablated eyestalk would be a good alternative, provided that the compound eye is removed prior to submission since it may contain a PCR inhibitor.

- Definition of a suspect case of WSD, according to OIE aquatic manual, for juvenile and adult shrimp is gross signs of WSD, for the shrimp at any life stage (larva to adult) is mortality and for shrimp and crab at any life stage (larva to adult) are hypertrophied nuclei in squash preparations of gill and/or cuticular epithelium; unusual aggregates in haemolymph by dark-field microscopy; inclusion bodies in histological sections in target tissues. Suspect cases should first be checked by PCR. If in a previously WSSV-free country/zone/compartment, PCR results are positive, then, they should be confirmed by sequencing. Histopathology, probes and electron microscopy also can be used to confirm the case.
Laboratory methods

Diagnostic Laboratory methods that currently are being used are:

1- Rapid methods of presumptive diagnosis, with two approach available, a) Preparing Wet mounts, demonstrate the hypertrophied nuclei in squash preparations of the gills and/or cuticular epithelium, which can be stained or unstained, b) Preparing Smears, demonstrate WSSV aggregates in unstained smear preparations of haemolymph by dark-field microscopy. This is the simplest of the microscopic techniques and is recommended for people with limited expertise in WSSV. The aggregates appear as small reflective spots of 0.5 \( \mu m \) in diameter.

2- Histopathology, with preparing fixed sections that demonstrates pathognomonic inclusion bodies in target tissues. The sections must be examined by light microscopy for the presence of moderate to large numbers of hypertrophied nuclei with eosinophilic to basophilic central inclusions surrounded by margined chromatin in tissues of ectodermal and mesodermal origin. The best tissues for examination are the subcuticular tissues of the stomach, cephalothorax or gill.

3- In situ DNA hybridization, by using WSSV-specific DNA probes with histological sections to demonstrate the presence of WSSV nuclei acid in infected cells.

4- Bioassay method, that will confirm the presence of a pathogenic virus, but does not identify the specific virus. Therefore, bioassay must be used in conjunction with laboratory tests to confirm the identity of the virus. If SPF shrimp are available, the bioassay method is based on Nunan L.M. et al. (1998) and Durand S.V. et al. (2000), is suitable for WSSV diagnosis recommended by OIE aquatic manual.

5- Transmission Electron Microscopy (TEM). Demonstrates the virus in tissue sections or in semi-purified negatively stained virus preparations (e.g., from haemolymph).

6- Antibody-based assays, There are various immunological assays including western blot analysis, immunodot assay, indirect fluorescent antibody test (IFAT), immunohistochemistry (IHC) or enzyme linked immunosorbent assay (ELISA) to detect WSSV by using both polyclonal and monoclonal antibodies raised against either the virus or a recombinant viral structural protein. Antibody-based methods can be fast, convenient and applicable to field use, but as they have only about the same sensitivity as 1-step PCR, they are recommended only to confirm acute WSD.

7- Polymerase Chain Reaction test (PCR). Two-step PCR test is the preferred recommended test by OIE aquatic manual. The protocol described in OIE aquatic manual is from Lo C.F. et al. (1997), and is recommended for all situations where WSSV diagnosis is required. A positive result in the first step of this standard protocol implies a serious WSSV infection, whereas, when a positive result is obtained in the second amplification step only, a latent or carrier-state infection is indicated. Alternative assays have also been described, but are not recommended unless they have first been compared with the protocol described there.

Many countries have developed the PCR test by designing primers from the virus strain in their country in order to prepare a more specific diagnostic kits for detection of WSSV. Iranians as well, have designed such kit by using a designated primer originally from VP24 protein of the virus in the diseased shrimp.

PCR commercial kits are available for WSSV diagnosis and are acceptable provided they have been validated as fit for such purpose. Please consult the OIE Register for kits that have been certified by the OIE (http://www.oie.int/vcda/eng/en_vcda_rejstrate.html).

8- DNA Sequencing of PCR products. For confirmation of suspected new hosts of WSSV, the DNA fragment amplified from the two-step nested diagnostic PCR should be sequenced. The cloning and sequencing protocols described in OIE aquatic manual are according to Claydon K. et al. (2004).

Picture: M. Afsharnasab, Outbreak in Khuzestan province, Iran, 2008. The designated primer, originally by VP24 with 414 bp from WSSV is used in the PCR test.
## Comparison of WSSV targeted surveillance and diagnostic methods

<table>
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<th>Method</th>
<th>Trageted surveillance</th>
<th>Presumptive diagnosis</th>
<th>Confirmatory diagnosis</th>
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<tbody>
<tr>
<td></td>
<td>Larvae</td>
<td>PLs</td>
<td>Juveniles</td>
</tr>
<tr>
<td>Gross signs</td>
<td>D</td>
<td>D</td>
<td>C</td>
</tr>
<tr>
<td>Bioassay</td>
<td>D</td>
<td>D</td>
<td>D</td>
</tr>
<tr>
<td>Direct LM</td>
<td>D</td>
<td>D</td>
<td>C</td>
</tr>
<tr>
<td>Histopathology</td>
<td>D</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>Transmission EM</td>
<td>D</td>
<td>D</td>
<td>D</td>
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<tr>
<td>Antibody-based assays</td>
<td>D</td>
<td>D</td>
<td>C</td>
</tr>
<tr>
<td>DNA probes in situ</td>
<td>D</td>
<td>D</td>
<td>C</td>
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<tr>
<td>PCR</td>
<td>D</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>Sequence</td>
<td>D</td>
<td>D</td>
<td>D</td>
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</table>

PLs = post larvae; LM = light microscopy; EM = electron microscopy; PCR = polymerase chain reaction.

A = the method is the recommended method for reasons of availability, utility, and diagnostic specificity and sensitivity;
B = the method is a standard method with good diagnostic sensitivity and specificity;
C = the method has application in some situations, but cost, accuracy, or other factors severely limits its application;
D = the method is presently not recommended for this purpose.

Source: modified from OIE Aquatic Manual 2009
PCR result of assessing the prevalence of WSD (outbreak 2005) in different aquaculture zones of Boushehr province, in Iran.

Results from Nested PCR of WSSV detection in different aquaculture zones of Boushehr province, Iran, 2005
(M = marker, 1,2,3,4,5,6,8,9,10,11 positive samples different aquaculture zones. 7 negative sample (Water for control), 12, 13 and 14 control standard in high, moderate and chronic level).
Source: Dashtyarnasab & Yeganeh.
Differential Diagnosis

- The clinical gross signs observed during outbreaks of WSD are nonspecific. Therefore, the diagnostician must consider any rapidly increasing mortality in a shrimp pond as potentially being due to infection by viruses, including WSSV. To aid in differential diagnosis, key features of the 4 major viral diseases known to be capable of causing massive mortalities in one or more of the penaeid species farms are compared in Table 5.

- Taura syndrome virus (TSV) has caused serious commercial losses only in juvenile to adult L. vannamei (Pacific white shrimp) in the Latin Americas farms and more recently in Asia. Infectious hypodermal and haematopoietic necrosis (IHHN), caused by IHHN virus (IHHNV), causes severe mortalities in farmed, and possibly wild, P. stylirostris (Pacific blue shrimp).

- Although P. monodon and P. japonicus are susceptible to infection, there are no reports of IHHNV-related disease in these species. Massive mortalities in individual shrimp ponds unrelated to disease events are rare, but can follow equipment failure or serious management errors (e.g. miscalculating chemical concentrations) as well as exposure to environmental toxins such as pesticides. However, such causes can usually be identified.

- Causes of more moderate mortalities, such as poor pond environmental conditions and subsequent bacterial infections in the shrimps, can usually be identified by inspection of pond records and examination of representative moribund animals, using histopathology and microbiology if necessary. Of particular note is the description of bacterial white spot syndrome in farmed shrimps, in which white spots macroscopically resembling those induced by WSSV are visible in the cuticle.

- Exposure to high alkalinity has also been associated with formation of white spots unrelated to WSSV infection or bacterial colonization. Neither of these non viral white spot conditions is associated with significant mortalities in affected shrimps.

- In summary, a provisional diagnosis of WSD is justified in the case of a disease outbreak in farmed shrimps characterized by high and rapid mortalities, white spots and/or red body discolouration on moribund animals, and demonstration using histopathology of eosinophilic to basophilic intranuclear inclusions in subcuticular epithelial cells. PCR and other tests can be used to confirm the diagnosis and rule out other possible aetiologies.
Differential diagnosis of virus-induced mortalities that may occur in 4 major viral disease in Penaeid farms

<table>
<thead>
<tr>
<th></th>
<th>White spot disease(in all farmed species)</th>
<th>IHHND(in <em>L.vannamei</em> and <em>P.stylirostris</em>)</th>
<th>Taura Syndrome(in <em>L.vannamei</em>)</th>
<th>Yellow Head disease(in <em>P.monodon</em>)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage of grow out</td>
<td>All</td>
<td>All</td>
<td>Usually 2–6 wks post stocking</td>
<td>Usually 7-10 wks post stocking</td>
</tr>
<tr>
<td>Mortality</td>
<td>High, rapidly Increasing, to 100% within a few days in acute phase</td>
<td>High, rapidly increasing in <em>P.stylirostris</em> within a few days, but in <em>L.vannamei</em> is as reducted growth and defects</td>
<td>Moderate in the peracute and acute phases</td>
<td>High, rapidly increasing to 100% within a few days</td>
</tr>
<tr>
<td>External appearance</td>
<td>Usually white spots embedded in cuticle of the carapace and body or red discouloration in body</td>
<td>White spot only in the 3 to 6 segment of the body</td>
<td>Acute phase: general red discolouration, especially tail fan</td>
<td>Often yellowish cephalotorax and general pale discoloration</td>
</tr>
<tr>
<td>Organs showing virus-induced necrosis</td>
<td>Subcuticular epithelium, connective tissue, gills, lymphoid organ</td>
<td>Gills, lymphoid organ, epidermis and hypodermis, ganglion, connective tissue, intestine, nerves</td>
<td>Subcuticular epithelium, connective tissue, gills</td>
<td>Subcuticular epithelium, gills, lymphoid organ</td>
</tr>
<tr>
<td>Inclusion bodies</td>
<td>Intranuclear; Initially eosinophilic (Cowdry type A) Then basophilic</td>
<td>Intranuclear; eosinophilic (Cowdry type A)</td>
<td>Intracytoplasmic; initially eosinophilic, then basophilic</td>
<td>Intracytoplasmic basophilic</td>
</tr>
</tbody>
</table>

Source: adapted from AQUAVET PLAN 2005- information: Shrimp Viral disease by M.Afsharnassab 2007.IFRO
Principals of Prevention and control

- Application of the principles of biosecurity is normally considered only for dangerous pathogens like WSSV, which are **highly virulent, infectious, untreated, vertically transmitted**, have a **diverse host range**, and **threaten the very survival of the industry**.

- The principles of biosecurity should be considered to **keep the pathogen not only out of the culture environment** but also **out of the country** and **the region**. For example, Australia has successfully kept many pathogens, including WSSV, out of the country by adhering to strict principles of biosecurity, quarantine and health certification.

- It is possible **to prevent WSD outbreaks** despite the **presence of the pathogen**.

- **Farm level of Biosecurity** should be conducted by farmers with the help of governmental authorities, but the **zone/country level** of it should be carried out only by governments before the outbreak as prevention or at the time of outbreak as control.

- **Farm level Biosecurity of WSSV**
  - Preventing the entry of WSSV into Hatcheries and Farms
  - Monitoring the health status of the shrimp population,

- **Disease outbreak and control**
Farm level biosecurity of WSSV

- Preventing the entry of WSSV
  Epizootologically speaking, likely pathogen carriers of WSSV, include:
  - Infected hosts (e.g. seed, broodstock, vectors, intermediate hosts, reservoir hosts),
  - Non-host biological carriers (e.g. birds, insects, other predators, human beings) and,
  - Fomites (e.g. water, vehicles, buckets, shoes, nets, clothing).

  The carriers can enter the culture system through waterborne, airborne and overland transport routes.
  - Waterborne transport may include contaminated water (e.g. pond effluents, processing plant effluents) and natural hosts in water.
  - Airborne transport (e.g. migratory birds, insects, wind) of pathogens is a concern in open farming systems without cover.
  - Overland transport (e.g. human beings, animals, vehicles, farm equipment) is the most common route of introducing the pathogen to the culture system.

  There are 2 critical points for preventing the entry of virus:
  1- Hatcheris
  2- Farms
Preventing the entry of WSSV in to Hatcheries

Main summarized preventive measures in hatcheries are as below:

- Using Specific Pathogen Free (SPF) and genetically improved (selective breeding method) brood stock should be the first priority to produce post larvae

- Screening of virus throughout the hatchery cycle from broodstock to post larvae prior to stock in cultured pond by two-step PCR test.

- Maintain the proper quarantine under a biosecurity principles during hatchery productions such as: implementing good sanitary practices, treating water before use, Optimizing stocking density of larvae and maintain good water quality, Treating hatchery effluent.
Preventing the entry of WSSV into Farms

- **Pond preparation** to eliminate pathogens and their carriers;
- **Water treatment** in reservoirs to inactivate the free virus and kill virus carriers,
- **Water filtration** using fine filters to keep carriers out,
- **Closed systems** to avoid contamination from source water,
- **Reduced water exchange** to minimize the entry from source water and even changing the water source (e.g. ground water) if required.
- **Preventing overland transportation of virus** by crab fencing, perimeter fencing, foot dips, wheel dips, and disinfection programmes.

The available evidence suggests that it is possible to minimize the entry of WSSV to the pond/farm if not totally prevent it.
Monitoring the health status of the shrimp population

- Beside the prevention, monitoring the health status of shrimps at any stage of life in grow-out farms is also vital by using double step PCR test on the samples collected by authorized technicians of veterinary organization of each county through their targeted surveillance program for WSSV.

- Early detection of the virus presence in its latent form in the population of a farm and subsequent controlling practices can minimize the impact of the virus and its probable outbreak, through taking intelligent decisions regarding continuing farming despite of the presence of pathogen by implementing special practices, or conducting emergency harvest or even destroying the stock. Such approaches can prevent the pond from becoming a pathogen farm and a risk to other ponds.

- It should be noticed that all these biosecurity principles would only be correctly implemented if there is a high level of adoption of Better Management Practices (BMPs) including concept of biosecurity among the farmers. The experiences of NACA in this regard in India and Vietnam should be considered seriously by the other countries. Awareness and capacity building of farmers on farm-level biosecurity concepts should be taken up on priority. System specific and cost-effective, better management practices (BMPs) incorporating principles of biosecurity should be developed, demonstrated and validated.
Disease outbreak & its control

- **Quick response** and **damage control** should be the **only post outbreak goal** and would prevent the spread of the disease in the farm or to other farms in that aquaculture zone or to the natural environment.

- Once the **outbreak occurred**, the farm becomes the source of pathogen; 1) Isolation of the farm, 2) avoiding movements onto and off the farm, 3) removal of infected hosts and 4) disinfection programs will help contain the spread of the pathogen.

- Co-operative effort through governmental authorities should include **surveillance, early warning, co-ordination of harvest and water exchange schedules** of contaminated ponds and **processor co-operation** to ensure that processing wastes are not threats to other ponds. In fact, a large percentage of shrimp harvest in WSD affected areas is premature (emergency harvest) and the harvested shrimp will have heavy loads of the virus. Transportation and processing of these shrimp pose a biosecurity threat.

- **Controlling and zoning for WSD may be difficult.** Several surveys have shown that shrimps can carry WSSV infections below the current level of detection available with a nested PCR test.

- **Under stress**, such as occurs during spawning in hatcheries, viral replication can occur, with infection reaching a level where it can be detected by PCR.

- Thus, **in the absence of stress**, latently infected shrimp populations may become established and could be very difficult to detect. **Reservoirs** of infection could become established in the environment in any of the species of crustacean that are susceptible to WSSV infection, and these reservoirs are unlikely to be successfully eradicated.
Overall Policy for Controlling WSD Outbreak

- Each country with shrimp farming industry should have its own tailored overall policy in the time of WSD outbreak. Here an overall policy has been briefly discussed based on Australian overall policy in the time of outbreak. Australia is at the moment free of WSD.
- In an outbreak of WSD, or if the white spot virus (WSSV) is detected, the choice of response policy should be decided by the director of fisheries and/or the chief veterinary officer (CVO) of the state or territory in which the outbreak occurs, following initial epizoological investigations.
- There are three possible response policies to be considered for WSD control:
  
  **Policy 1 - Eradication** with the aim of having the country returns to being free from WSSV;
  
  **Policy 2 - Containment, control and zoning** of the virus to areas with enzootic infection, prevention of further spread and protection of uninfected areas; and
  
  **Policy 3 - Control and mitigation of disease** if it is accepted that the virus will remain enzootic in the country.

- All these response policies involve the use of a combination of strategies, which may include:
  
  - *Quarantine and movement controls* on crustaceans and things in declared areas to prevent spread of infection;
  
  - *Destruction* of all clinically diseased or dead shrimps as soon as possible, to prevent further virus shedding;
  
  - *Decontamination* of facilities, products and things to eliminate the virus from infected farms and premises and to prevent spread of infection;
  
  - *Surveillance* to determine the source and extent of infection and to provide proof of freedom;
  
  - *Zoning* to define and maintain infected and disease-free zones; and
  
  - *Hygiene and biosecurity* measures aimed at mitigating the on-farm effects of WSD.
Eradications

*Eradication* may **not** be feasible if epizoological investigations determine that WSSV infection is **widespread across most or all of the shrimp aquaculture zones**, **has no controllable source** or is otherwise **unable to be contained**.

Eradication measures include:

- Establishment of specified areas — restricted, control, free;
- Quarantine and movement controls or restrictions on shrimps, other crustaceans, water and any other vectors (including materials and equipment) in declared restricted and control areas to prevent the spread of infection;
- Destruction and disposal of all clinically diseased shrimp,
- On-farm processing (for example, by cooking) of exposed or potentially exposed, but clinically normal prawns to prevent the spread of infection;
- Disinfection and safe disposal of **processing effluent and waste** (cooking water, shrimp heads and shells);
- Disinfection and safe disposal of pond water and decontamination of ponds, facilities, products, equipment, vehicles, boats etc to eliminate the virus from infected premises and to prevent spread;
- Use of farm perimeter barriers to prevent entry or escape of potentially infected wild crustaceans;
- Tracing and surveillance to determine the source and extent of infection and to provide proof of freedom from the disease; and
- A public awareness campaign to encourage cooperation from industry and the community.
Containment, Control & Zoning

Similar to Eradication, the feasibility of Containment, Control and Zoning will depend on farm management practices, the extent to which infection has already spread, and the location, distribution and migratory behavior of infected species. Measures for containment, control and zoning are similar to those for eradication, but will emphasize management of the disease in individual facilities. Procedures may include:

• **Zoning to define infected and disease-free** areas; A successful zoning strategy will rely on movement restrictions on exposed or potentially exposed prawns to prevent infection spreading to uninfected zones.
• **Quarantine and movement controls** or restrictions on shrimps, water and any other vectors (including materials and equipment) within the infected zone and to free zones;
• **Eradication of outbreaks in the free zone where feasible**;
• **Pond-level surveillance**, with destruction and safe disposal of any clinically diseased shrimps in the infected zone, followed by clean-up and disinfection;
• **Use of closed production systems**;
• **WSSV testing of broodstock and post larvae**;
• **Emphasis on high standards of hygiene** (including drying of ponds before restocking and disinfection of water before either use or release) and **biosecurity** (use of crustacean-proof land barriers and water filters and screening of incoming post larvae for WSSV);
• **Tracing and surveillance to determine the source and extent of infection**; and
• **A public awareness campaign** to encourage cooperation from industry and the community.

-In containment, control and zoning strategy, grow-out of exposed or potentially exposed, but clinically normal shrimps will be a standard practice within infected zones. The likelihood of clinical disease will be minimized through appropriate farm management practices. Spread of the disease will be prevented through farms maintaining a high level of hygiene and biosecurity.
Control & Mitigation

If infection is widespread, and there is evidence of widespread infection in wild broodstock populations (on those countries that rely on wild broodstock like Australia), Control and Mitigation of the disease in an industry-based program tailored for each country farming specification, is likely to be the most appropriate option. In a control and mitigation strategy, it will be the responsibility mainly of individual producers to manage the disease in their facilities using recommended measures by the responsible authorities to reduce the likelihood and severity of outbreaks. Producers may be encouraged to adopt current best practice through provision of enterprise-level standard operating procedures and quality assurance programs, leading to the eventual development of an accreditation scheme.

Measures for control and mitigation include:
- Pond-level surveillance, with destruction and safe disposal of all clinically diseased shrimps followed by clean-up and disinfection of affected ponds;
- Use of closed or partial recirculation production systems, as appropriate;
- WSSV testing of broodstock and post larvae;
- Emphasis on high standards of hygiene (including drying of ponds before restocking and disinfection of water before use or release) and biosecurity (including covering the ponds by net or similar to avoid birds’ access, the use of crustacean-proof land barriers and water filters); and
- Best-practice pond management methods to minimize stress and hence the risk of an outbreak during grow-out of covertly infected stock.

During grow-out, these shrimps must be treated and handled as infected populations. Restrictions on movements of prawns, people, vehicles and boats, and on market access for products, may be necessary to protect WSSV-free facilities or zones.
Prevention and Control in Iran

- Iran, with records of WSD occurrences in the past ten years seems to have implemented (according to aforementioned overall policy) kind of a containment, control and zoning policy, tailored to its shrimp farms’ specification which is an open semi intensive farming system, located in different aquaculture zones in its 4 coastal provinces in Northern coast line of Persian Gulf and Oman Sea.

- Sharing the experience of Iranians in several challenges with WSD, for the countries in the region who has shrimp aquaculture zones or planning to have, can be beneficial due to the similarity of the climate and culture environment and the fact that with presence of the WSSV in the northern shores of the Persian Gulf and Oman Sea, the probability of spreading of the virus through vectors especially those with migratory behaviors, to other parts of the region is very high.

- The responsible governmental authority in Iran in this regard is” Iran Veterinary Organization (IVO)” that has prepared a code of practice (Ref No.1388/44/01) (IVO official web site www.ivo.org.ir) in association of the Iranian Fisheries Organization (IFO) to be implemented in WSD outbreaks, valid at the time of writing this article.

- The policy is based on prevention of the disease according to principles of biosecurity with special attention on the importance of prevention of WSSV spread and management of the disease occurrence in order to minimize its impacts. In parallel to this, IVO has insisted on making reservoir pond as the main preventive structural needed part of the current open farming system, for treating the inlet water, through preparation and adoption of BMP (best management practices) guidelines in association of IFO, for the farmers. Also all the future established farms are planned to be equipped with such reservoirs as a rule.
1-Actions required:
- Quarantine and movement controls or restrictions on shrimps, water and any other vectors (including materials and equipment) within the infected zone and to free zones must be implemented by the resident IVO technicians. The details of the any permitted movement must be recorded.

- All the other farms in the infected zone must be under the surveillance and in case of reporting any mortality or clinical signs; detection for the causative agent must be carried out to define the nature of the problem.

Instructions for managing the diseased farm(s):
1- In case of detection of the WSSV in the first month after stocking by using Nested-PCR:
1-1-Farm shows clinical form of WSD:
- If the shrimps are lethargic or anorexic, show clinical signs of WSD with mortality, water inlet and outlet must be closed, Killing of the shrimp and disinfecting the water must be done by using 40PPM of Calcium Hypochlorite, and the carcass and the water should be discharge only after 7 days, All the remained decomposed shrimp as well as pond bottom dirt or remained in the filters must be collected, burned and buried with lime.

1-2-Farm does not show any clinical sign of WSD:
- By implementing high level of hygiene and Biosecurity together with appropriate farm management avoiding any stressful situation in the ponds, the culture can be continued.
2- In case of detection of the WSSV in the second month after stocking by using Nested-PCR:

2-1-The culture can be continued in association with avoiding any stressful conditions, if the shrimp do not show abnormal activity, are active, feeding well, exhibiting normal growth, do not have clinical signs of white spot and are not dying. In such cases, application of iodine 10% at 0.3-1 ppm (repeated application at 3-4 days interval), or formalin at 70 ppm (every day) or BKC at 1 ppm in grow-out ponds with WSSV and the nearby ponds is recommended. The function of these chemicals is to prevent the horizontal transmission of the virus among the shrimps in the pond.

2-2- If the shrimp health condition deteriorates, the shrimp stop feeding, shrimp show clinical signs of white spots and start dying, and single step PCR testing is positive, then:

- In case of having the farmed shrimp with appropriate size and weight for market, harvest must be done maximum within 24-48 hours after the confirmed report of disease, supervised by IVO technicians according to biosecurity principles, in such a way that no dead or alive shrimp or any aquatic creature could be released from the pond, and disinfection of the pond water must be done by using 40 ppm calcium hypochlorite and at least, leave for 7 days before discharging. All the remained of carcass or other materials must be collected and burned and buried with lime. The bottom of the pond must be disinfected by applying lime (1.5 Mt/Ha). Transportation and processing of such shrimp must be done under full supervision of the IVO technicians.

- In case that the shrimp is not at market size or the farmer does not have an intention for harvest, then it should be treated as section 1-1 with the regard that if some live shrimp were seen in the pond after applying Calcium Hypochlorite, the pond must be disinfected again with increased dose of 100ppm.
2- Special notes:

- Disinfection of all the equipment and materials in the pond must be done by spraying water solution of Calcium Hypochlorite (1600ppm) or if possible the equipment can be submerged in its solution (40ppm) for at least 3 days.
- Disinfection of vehicles especially the wheels must be done with spraying water solution of Calcium Hypochlorite (1600ppm),
- Disinfection of the workers clothing, shoes, boots must be done and it is better to use disposal gloves and foot covers in the next operations.
- Disinfection of the outlet water channel of the farm must be done with Calcium Hypochlorite (40 ppm).
- Disinfection of the pond bottom must be done when it is still wet with lime appropriately after testing the soil pH, and then should be left to be dried completely.
- The disinfected water of the pond must be discharged after at least 7 days, through the filters in order to minimize the release of dirt particles and their associated microorganisms in the environment. The filtered materials must be collected, burned and buried hygienically.
- Movement of workers, equipments, vehicles from the infected farm to other farms is forbidden.
- Entrance of feed trucks to infected farm is forbidden and the feed delivery must be done out side the infected farm and should be transported to infected farm according to all principles of hygiene and biosecurity.
- IVO technicians should have in place (for each farm) equipment and cloths etc. and it should not be taken out from infected farm before end of the control of the situation.
- All the sampling and transportation of the shrimp from the infected pond should be done subject to prior awareness and permission of IVO.
- The presence of the birds in the pond must be minimize to even zero by any means, and all the moribund shrimp near the edge of the pond must be collected regularly and burned and buried with lime.
- Even when disinfectant is used to kill the shrimps, the pond water and dead shrimp cannot be released soon after the death of shrimp, because the virus is viable for a longer time within the nuclei of deeper tissues of the shrimp body even after its death. Thus, the dead carcass must be kept in the pond for about 7 days to let the shrimp decay and to allow the virus to completely die by autolysis. Release of freshly killed shrimp into open water will result in the spread of the disease and its persistent in the same farm.
### White Spot Disease control measures in Iran during July-Dec from 2005-2009

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A=Applied, *= Notifiable disease, Qf=Precautions at the borders, M=Monitoring, Te=Screening, Gsu=General surveillance, Tsu=Targeted surveillance, Qi=Movement control inside the country, S=Stamping out, Sp=Modified stamping out, Z=Zoning, Vp=Vaccination Prohibited, V=Routine vaccination, T=Treatment, Cr=Control of wild life reservoirs, Cn=Control of arthropods.

Source: OIE website
Discussion

Current achievements and experiences in many countries has proven that it is possible to reduce impact of WSD and improve productivity and profitability of small-scale shrimp farms through disease control programs, specially at the time that there is no vaccination method developed against the disease despite of many researches that has been carried out in this regard.

These control programs should be developed and progressed by continuous update of the knowledge about the disease and its causative agent, providing access to science-based disease control principles as well as technical supports that enables farmers to adapt BMPs principles to their own circumstances, and promotion of local self-help groups as experienced by NACA in different countries to facilitate cooperation and communication of BMPs to a wider group of farmers, and to collectively address health management problems. In order to have a successful promotion in adoption of biosecurity principles, the approach should be implemented within a governmental institutional framework as a national program.

Farm level biosecurity programs should be system specific, cost-effective based on the socio-economic back ground and mindset of the farmers, also comprehensive and applied from site selection stage through to harvest.

In order to have intelligent decision making and designing preventive and post-outbreak programs, epizoological concepts should be considered.

Regional collaborations of countries in sharing their experiences can lead to collective approaches for controlling the disease, minimizing its impact on the farming industry and preventing the spread of the disease that will be a threat for the others in the region.
Acknowledgements

The author wish to thank the following Gentlemen for their kind support and encouragement in preparing this review;

- Dr. Ghazi Yehya, World Organization for Animal Health, Regional Representative for the Middle East
- Mr. M. Mabsout, Managing Director of Mabsout & Idriss SAL,
- Dr. M. Afsharnassab, Director of Division of Shrimp Health and Diseases, Iranian Fisheries Research Organization,
- Dr. A. Sepahdari, Director of Division of Fresh Water Aquatic Animal Health and Diseases, Iranian Fisheries Research Organization,
- Dr. R. Abdi, Head of the department of Aquatic Animal Health, Iran Veterinary Organization,
- Dr. A. Ghajari, Department of Aquatic animal Health, Iran Veterinary Organization.
- Dr. R. Bana Derakhshan, Deputy of Division of Shrimp and other Marine Aquatics, Iran Fisheries Organization.
- Mr. M. Hekmat Shoar, Head of Shrimp Aquaculture Bureau, Aquaculture Department, Iran Fisheries Organization.
- Mr. M. Jahanbani, Executive director of Nayband Gulf Aquaculture and Industry Co.
Thank You For Your Attention !