White Spot Disease

White spot syndrome

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Importance

White spot disease is caused by the white spot syndrome virus (WSSV) and causes high mortality in post larvae and juvenile shrimp (possibly close to 100% in only a few days); it's an acute disease and it can be transmitted horizontally or vertically (zooplankton, contaminated water, pond sediment and cannibalism). The manifestations of the disease tend to appear during the first 15-50 farming days in production ponds.

Stress is a fundamental factor in the disease development. A marked relationship between temperatures under 27°C and the appearance of the disease has been noted. Over 29°C, infected shrimp may become asymptomatic carriers. Over 32°C the virus reduces its activity dramatically, mitigating, or delaying mortality.

Other factors that cause stress and trigger the disease in WSSV-infected shrimp include, low levels of dissolved oxygen, extreme pH values, sudden changes in water quality, high levels of suspended solids, toxic substances in the water, unilateral ablation of the eye stalk (females), and spawning. In pls stressing factors include harvesting, packing, transportation, and acclimation.

It has been determined in some cases that white spot disease is related to a presence of opportunistic bacteria in shrimp hemolymph (bacteremia). This condition suggests that the toxins released by abundant bacteria populations infecting the shrimp, predisposes organisms to WSSV outbreak due to septic stress. The hypothesis that supports this situation proposes that this toxemia causes immunosuppression and/or activates viral replication, causing tissue damage and clinical signs within 24 to 48 hours of the acute phase of the bacteriosis.

WSSV Virus Characteristics

- Family Nimaviridae
- Genus *Whispovirus*
- Baculovirus, with double-stranded DNA
- Ovoid, ellipsoid or bacilliform shape
- Trilaminar membrane
- Virions of 80-120 to 250-380 nm in size
- Presence of an appendix flagella-like
- Its genome is approximately 290 kbp
- In culture ponds, it is viable in water for 12 days and in sediment for 3 days
- Virus losses infectivity on sun-dried pond bottom after 19-21 days and on wet bottom after 35-40 days
- It becomes inactive after 120 minutes at 50°C and 1 minute at 60°C
- Its replication cycle takes 20 hours at 25°C

Geographic Distribution

White spot syndrome virus was first reported in 1992 in northeast Asia and spread quickly in most shrimp producing countries in Asia and the Indo-Pacific, including China, Thailand, Japan, Korea, Indonesia, Malaysia, Taiwan, Vietnam, and India.

The first case of WSSV in the West was confirmed in 1995 in Texas (USA) on a *Penaeus setiferus* farming facility. It is possible that this outbreak was produced by contamination from a shrimp processing factory used for processing shrimp imported from Asia.

In 1999 WSSV was reported in Panama, Nicaragua, and Honduras, and appeared shortly thereafter in the other Central American countries, Mexico, Colombia, Ecuador, Peru, and Brazil where it was detected in *P. vannamei* farming ponds. Later on, it was detected in Madagascar, Mozambique and Saudi Arabia, spreading after to other regions.

Today WSSV is found in countries of Asia, the Middle East, Americas, Oceania, and the Mediterranean, with areas free of infection in these regions.

The Center for Food Security & Public Health

Institute for International Cooperation in Animal Biologics

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World Organisation for Animal Health Founded as OIE

USDA

Affected Species

Natural infections have been observed in wild and farmed shrimp species that include *P. monodon*, *P. japonicus*, *P. chinensis*, *P. indicus*, *P. merguiensis*, *P. setiferus*, *P. stylirostris*, and *P. vannamei*. Tests performed under laboratory conditions, showed that a WSSV strain from Thailand is highly pathogenic for *P. vannamei*, *P. stylirostris*, *P. aztecus*, *P. duorarum*, and *P. setiferus*.

No significant natural resistance has been observed in any penaeid shrimp species. Although, families of *P. vannamei* from a breeding program in Panama shown greater rates of survival in WSSV challenge tests performed at the experimental facilities of the University of Arizona (USA).

Species that fulfill criteria according to Chapter 1.5 of the WOAH Aquatic Manual for including as susceptible WSSV species are currently under study

Main Sources of WSSV Contamination

The main virus contamination factors are:

- Shrimp and post larvae infected with WSSV
- Marine animals other than crustaceans that are virus carriers
- Viral particles disseminated in water and bottom
- WSSV-positive processed products
- Wastewater from shrimp processing plants
- Equipment, vehicles, human activities
- Marine or freshwater decapod crustaceans

Clinical Signs

The following are clinical signs that can be observed during WSSV outbreaks:

- Anorexia (loss of appetite)
- Empty digestive tract (stomach and gut)
- Motor disorders (nervous signs)
- Expanded chromatophores
- Red uropods
- Soft texture (exoskeleton and sometimes the abdominal muscle)
- Erratic swimming
- Loss of escape reflex
- Lethargy
- The cuticle detaches easily during premolt
- Appearance of white spots of up to 2.0 mm under head carapace (calcium carbonate deposits), giving the disease name. They may or may not be present during WSSV outbreak.
- In populations where the virus has just entered, the appearance of these signs precedes high mortality rates that can reach 100% in the first 3-10 days after the appearance of the first clinical signs.

When clinical signs are present, shrimp must be fixed for histopathology using Davidson's solution. Part of the samples must be fixed for PCR in 95% ethanol in order to complement diagnosis by confirming genomic virus presence. Samples can be also tested by using a quick field-test based on immunochromatography, which is commercially available for WSSV and give results within 10 minutes. This test is highly specific, and its sensitivity is equivalent to a one-step PCR test.

Diagnosis

Clinical diagnosis

White spot syndrome can be suspected when shrimp present some of the already mentioned clinical signs and/or when they look lethargic, with reddish or whitish discoloration, slow swimming or staying immobile near the pond walls. When trying to capture them by hand, they show no resistance due to the loss of escape reflex.

WSSV affected shrimp may present soft shell and flaccid abdominal muscle (shell must be hard except during molting). Stomach and gut are visibly empty (without feed) and dark chromatophores tend to expand. Some shrimp also have red uropods. In *P. vannamei* shrimp, white spots are sporadically observed under the head cuticle when carapace is lifted using the nail and observing with a dark background.

Differential diagnosis

Differential diagnosis of WSSV includes Systemic Vibriosis, Yellowhead syndrome, and Taura syndrome (acute phase). Moreover, it includes other possible conditions in which anorexia and reddish discoloration are present, such as chemical or biological toxicity.

Laboratory Analysis

White spot disease should be diagnosed only from sick shrimp captured in ponds (or water bodies) where mortality is observed or when there are shrimp with suspicious clinical signs.To determine if WSSV is the cause of an outbreak, sick shrimp must be fixed in Davidson solution in order to be studied by histology. This diagnostic analysis is confirmatory if Cowdry “A” inclusion bodies (intranuclear) are observed in the cuticular epithelial cells (mainly in the stomach), gills, hematopoietic tissue, connective tissue, and antennal gland. There is a similarity between inclusion bodies due to WSSV and those produced by the infectious hypodermal and hematopoietic necrosis virus (IHHNV) and must be confirmed with in situ hybridization or PCR. When disease by WSSV is advanced, these inclusion bodies look larger and basophilic.

As mentioned before, field tests based on immunochromatography offer high sensitivity and they are highly specific for WSSV allowing a colorimetric detection of the virus in suspicious shrimp tissues like pleopods and gills. Immunochromatography commercial kits for WSSV detection are similar to human pregnancy tests. Other immunodiagnostic tests include monoclonal antibodies, but their commercial application is less frequent.

For genomic detection of WSSV in shrimp tissues, hemolymph, gill, or pleopod samples are generally used. Molecular tools that can be used for these diagnostic tests include *in situ* hybridization, Dot Blot hybridization, and PCR (one-step PCR, nested-PCR, real-time PCR, LAMP and iiPCR).

Laboratory Sampling

Histopathology

For this type of technique, selected shrimp must be alive, visibly sick and showing the clinical signs described above (preferably moribund organisms). For WSSV disease diagnosis, 5-10 sick shrimp are enough for histology studies and they must be fixed through injection with Davidson fixative solution, which preparing formula is as follows: 95% absolute ethanol: 33%, 37% formaldehyde: 22%, glacial acetic acid: 11.5%, and distilled water: 33.5%. Shrimp injection must be plenty in cephalothorax (head) and especially in hepatopancreas which must be the first injected organ. Lateral injections in the abdomen can be done in big organisms. They should then be submerged in Davidson for 24 hours (larvae or post larvae), 48 hours (juveniles or pre-adults), or 72 hours (adults - broodstock). After proper time in Davidson's solution, fixative must be drained and replaced with 70% ethanol. It must be repeated 24 hours later. If samples are not yet processed within next 2 weeks, 70% ethanol must be replaced again to avoid tissue´s acidification. The fixing process should always be performed using gloves, protective goggles, and in an open, well-ventilated place to avoid inhaling Davidson's vapors. This fixative solution can be carcinogenic due to its formaldehyde content.

The routine histological process for shrimp samples takes 12-hour for dehydrating, paraffin embedding, microtome sectioning (3 micron), and staining (Hematoxylin and Eosin (H&E) is the routine staining). The shrimp Pathologist must review histological slides under the microscope in an ascendent way using objectives of 4X, 10X, 40X and 100X (last one requires immersion oil). The complete histopathological process, from the fixing of the shrimp to the interpretation of histological slides under the microscope and elaboration of the diagnostic report, can take from 5 to 7 days under regular conditions.

Immunochromatography

This technique is performed using a commercial kit that includes all the materials and reagents necessary to realize the test. Samples for this technique must be sick shrimp presenting clinical signs suggestive of White spot disease. Shrimp samples required for this test are pleopods. Depending on the shrimp size, it may be necessary to use 2 or more (up to 10 for small juveniles). In some cases, a pool of pleopods from 2 to 5 shrimp from one pond can be processed as a sample. It's usual when it´s only important to know if the pond is positive or not to WSSV infection and no prevalence data is needed. Test setup takes 10 minutes and obtaining results other 10 minutes for a total test time of 20 minutes.

This test has the advantage of not requiring sophisticated laboratory equipment or supplies (scissors or forceps and tap water with soap to wash them are the only needed items). This test offers quick and reliable results, giving farm technicians a quick response for quick pond decisions.

Molecular Tests.

For the detection of WSSV there are commercial kits based on gene probes and PCR techniques. Gene probes include Dot blot hybridization and *in situ* hybridization tests. Regarding Dot blot and PCR, both use shrimp pleopods or hemolymph for WSSV genomic detection. For Dot blot technique shrimp tissue is grinded with a lysis buffer to break cells and expose the viral DNA, which remains free in the extraction solution. This test is performed on a Nylon or Nitrocellulose membrane and is based on the detection of the viral genome with a specific probe, joined to a marked enzyme that produces a colorimetric reaction that gives as a result dark blots macroscopically visible. The intensity of the blots is proportional to the viral load contained in the sample. Based on that, the darker the blot, the greater the quantity of viral material, making Dot blot technique a semi quantitative test. Dot blot tests are usually initiated in the afternoon, incubating overnight, and revealing results next morning. Results are ready about 48 hours after samples have been submitted to the laboratory, including report writing.

In situ hybridization is equivalent to a Dot blot test, but performed directly on a previously dewaxed, unstained histological section from a sick shrimp previously fixed in Davidson and processed according to the routine histological protocol. The results are given based on the colorimetric reaction observed under a microscope view in the cells where the virus is present. Positive (infected) cells will show a brown or dark blue (almost black) nuclei indicating the presence of WSSV viral particles. The complete procedure can take from 48 to 72 hours including slides review and diagnostic report writing.

About the PCR technique for WSSV detection, it has several possible formats that include one-step PCR, nested-PCR, real-time PCR (or qPCR), LAMP and iiPCR. These tests use hemolymph, pleopods or gills. For post larvae 5 to 20 sick organisms are used (depending on size), removing previously their eyes because they can interfere with PCR reaction. The samples are submitted to DNA extraction and then a small amount of DNA is used to prepare the amplification solution, which also includes primers, Taq polymerase, buffer and deoxynucleotides. The genomic amplification is performed in a thermocycler (PCR machine) and takes from 1 to 2 hours. The amplified product (amplicon) is migrated in an agarose gel through electrophoresis for 20 to 30 minutes. DNA bands are revealed on a UV transilluminator, making the bands visible due to the presence of Ethidium Bromide attached to DNA in the gel.

Positive samples are confirmed by observing a shiny purple band in the gel. Samples where no band is present can be reported as "non detected" for WSSV. The complete

process can take from 4 to 8 hours if work is done in an exclusive, uninterrupted manner. Regular laboratories usually send results after 48 hours of receiving the samples. PCR technique requires sophisticated facilities, equipment, and reagents, as well as qualified and well-trained staff.

Recommended Measures when White Spot Disease is Suspected

Notifying the Authorities

White spot disease is a disease of penaeid shrimp that must be notified to the World Organisation for Animal Health (WOAH, former OIE). The requirements for notifying the disease to the WOAH for member nations and import/export guidelines, can be found in the WOAH Aquatic Animal Health Code. Veterinarians that detect a case of white spot disease should follow national and/or local guidelines for notification and the applicable diagnostic tests. Nevertheless, it should be pointed out that this disease is listed as endemic in the Americas, where it is widely distributed in countries that produce farm-raised shrimp, with presence of WSSV carriers that don't show any of the clinical signs.

Control Measures for White Spot Disease

After WSSV has been introduced in a country, nauplii and post larvae should not be purchased from laboratories that could be or are infected with this virus. It is likely that iodine and water baths can remove and destroy the virus from the eggs, nauplii, and post larvae on infected shrimp. It is essential for this process to be consistent. Farms should maintain robust biosecurity measures and examine every lot of animals before purchase and stocking in ponds.

Sensible and specific diagnostic tests must be used to detect and track WSSV. These usually consist of DNA-based technologies, such as PCR and in situ hybridization (ISH).

It is important to purchase post larvae that have been analyzed using PCR after having undergone and passed the stress test. This test consists in submitting a small group of animals to sudden changes in salinity, rapidly going from 32 ppt to 0 ppt and, after a few minutes, returning them to salinity of 32 ppt and determining the survival (%). Pond stocking at shrimp farms must be done using post larvae with no viral load, or extremely low viral loads.

Although it is known that the use of formalin helps to eliminate the weakest post larvae, farms should never stock postrave known to be carriers of the WSSV virus. It has been observed that the virus shows no pathogenicity in *P. japonicus* until Pl-6 stage. Thus it´s crucial to test by PCR post larvae after that stage.

Stress in shrimp populations must be reduced whenever possible following these suggested recommendations:

- Increase acclimation time before stocking
- Use non-specific immunostimulants (NSIS) and diets fortified with minerals and vitamins to raise stress tolerance
- Acquire and stock post larvae at times of the year when they will not experience high stress due to sudden changes in temperature and salinity
- Use high-quality diets and continue using NSIS throughout the entire production cycle
- Implement production cycles with low stocking densities
- Vector monitoring and control to avoid WSSV introduction in farming ponds
- Perform PCR tests on phytoplankton and zooplankton samples from the pond before stocking to detect the presence of WSSV. Stocking must be avoided in ponds that are positive for the virus

There are conditions that trigger White spot disease and that cannot be controlled. One of the main problems that shrimp producers face during farming cycle is sudden weather changes that can occur during the rainy season. Abrupt changes in temperature and salinity have been associated with the appearance of many outbreaks of the disease. Inasmuch as the disease moves from one zone to another, the viral load will increase in the environment surrounding the farm, to the point that the virus is always present in the neighborhood. Ideally, sick shrimp would be eliminated once WSSV is confirmed, in order to avoid the accumulation of high viral loads in the environment. Unfortunately, this procedure is not practical. Shrimp are usually harvested at any cost when needed, even if the population is still too small to garner a market price that will cover production costs. As far as possible, losses should be reduced, and the spread of the virus should be minimized within and between farms.

It is relevant to mention that due to the endemic condition of the disease in many countries, it's possible to find shrimp with high viral loads showing no clinical signs. In these cases, the disease expression depends on the susceptibility of the infected species, the population's resistance to the virus and sudden environmental parameter changes. Therefore, a viral load by itself does not cause disease or mortality in all susceptible species. Blue crabs (*Callinectes sapidus*) can contain up to 100 times more viral load of WSSV in their tissues than *P. vannamei* shrimp. This makes Blue crabs highly infective organisms if consumed by healthy penaeid shrimp.

As effective methods to obtain the inactivation of the white spot syndrome virus, the WOAH recommends the following alternatives:

- Heat: 55°C for 90 minutes or 70°C for 5 minutes
- pH: pH 3 for 60 minutes or pH 12 for 10 minutes
- Ultraviolet light (UV): 9.30 x 105 µWs/cm2
- Ozone: 0.5 µg mL-1 for 10 minutes
- Chlorine: 100 ppm for 10 minutes
- Iodophors: 100 ppm for 10 minutes

Public Health

Humans are not prone to contracting infection by white spot syndrome virus because this is not a zoonotic disease.

Internet Resources

World Organization for Animal Health (WOAH)

WOAH Aquatic Animal Health Code

WOAH Manual of Diagnostic Tests for Aquatic Animals. Infection with white spot syndrome virus.

Control and Management of the White Spot Syndrome Virus (WSSV). The Fish Site.

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