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Occurrence of white spot syndrome virus in shrimp culturing waters and its brunt in specific pathogen free *Litopenaeus vannamei* with particular allusion to molecular verdicts

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ABSTRACT

Objective: To detect the water samples and shrimp samples in white spot syndrome virus (WSSV) affecting and non-affecting zone. **Methods:** A total of 12 samples specific pathogen free *Litopenaeus vannamei* (*L. vannamei*); adult shrimp and larvae were randomly collected. Their genomic DNA was isolated and subjected to PCR. Histopathological identifications were carried out, and the hematopoietic tissues with basophilic intranuclear inclusion bodies characteristic were observed in moderate WSSV infected *L. vannamei*. **Results:** The PCR analysis showed the appearance of a prominent band from the PCR amplified product of WSSV-DNA at internal control band 848 bp at non-infected areas. Although low infection positive bands (20 copies) were shown at 296 bp continued from initial stage of the infection region. On a moderate and ascetic level were observed as 650 bp and 910 bp (200 and 2 000 copies), during the severe out break periods. The gill epithelial cells were edematous and nuclei were hypertrophied with basophilic inclusions, but no pathological changes or hypertrophied nuclei were observed in any of *L.vannamei* tissues in WSSV uninfected region. The Intranuclear inclusion bodies characteristics of high level of WSSV infection presented in the gill region. **Conclusions:** The present study is significant, which investigated the level of WSSV transmission from the infected tiger prawn *P.monodon* to SPFL. vannamei in the WSSV impact region of Tamil Nadu coastal waters.

1. Introduction

The crustacean farming industry has been suffering solemn problems and enormous economic losses from an outbreak of white spot syndrome virus (WSSV) since 1993[1]. WSSV is an acute pathogen and can infect numerous crustaceans, including shrimp, crab, and lobster[2,3]. Shrimp cultivation is often affected by outbreaks of deadly infectious diseases caused mainly by viruses[4]. In the maintenance of substantial production of farmed shrimp, an understanding of the shrimp immune system would allow for the development of management strategies to control virulent or problematic pathogens encountered on shrimp farms[5]. WSSV, the pathogen of shrimp white spot disease, is a rod-shaped enveloped dsDNA virus belonging to Nimaviridae family[6]. This virus can infect many kinds of marine and freshwater crabs and shrimp[7]. The genome size of this virus is about 300 kb, which contains about 184 open reading frames (ORFs). Thirty nine ORFs are structural proteins and 22 of them are envelope proteins, such as VP15, VP19, VP24, VP26, and VP28[8].There are a lot of studies for preventing and controlling shrimp 65 WSSV infection, such as improvement of environmental conditions, induction of non-specific antiviral response with antivirus drugs or immunostimulants[9,10], neutralization antibodies[11-13] and suppression of virus by RNAi technology[13,14].

In many countries, aquaculture is a major thrust area which improves community progress, food security and poverty mitigation and as the source of livelihood[15]. *Litopenaeus vannamei* (*L. vannamei*) is the most important shrimp species in terms of aquaculture production. Other important species are *Penaeus monodon* (*P. monodon*), *Penaeus chinensis, Penaeus merguiensis, Penaeus japonicus and F. indicus*. *L. vannamei* has several advantages compared to other cultured species. These include the availability of specific pathogen free (SPF) and specific pathogen resistant (SPR) strains, a higher growth rate, suitability to be higher stocking density, tolerance to a wider range of temperature and salinity, a lower protein

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requirement in diet, easier to breed and higher survival in larval rearing. These aspects could explain the increasing preference to culture this species. This information will expand our knowledge and may contribute to develop effective prophylactic or therapeutic measures. Recent studies have shown that viruses have the ability to manipulate the life histories and understanding of the hosts in remarkable ways, challenging our understanding of the almost invisible world of viruses[16].

Conventional identification of virus involves isolation of viral genome followed by nested PCR, DNA-based detection and symptomatic methods have the potential for widespread application of in aquaculture[17]. Efforts to overcome these problems have led to the development of immunoassay and DNA-based symptomatic methods, including fluorescent antibody tests (FAT), enzyme-linked immunosorbent assays (ELISA), radioimmunoassay (RIA), in situ hybridization (ISH), dot blot hybridization (DBH) and polymerase chain reaction (PCR) amplification techniques. The use of DNA-based methods derives from the premise that each species of pathogen carries unique DNA or RNA sequences that differentiate it from other organisms.

The present study investigated the level of WSSV transmission from the infected tiger prawn *P. monodon* to SPF *L. vannamei* in the WSSV impact region of Tamil Nadu coastal waters, in connection to PCR detection and histopathological observation. And survey of WSSV affecting and non-affecting zone and detection of environmental water samples with appropriate infected shrimp sample were carried out. Detection limitation is followed with molecular based diagnosis dealing with nested PCR and histopathology analysis. In an effort to reduce the accidental introduction of non-native organisms (including pathogens), guidelines has to be established to assist governments and the private sector in importing non-native aquatic organisms for fishery or aquaculture use in a responsible manner.

2. Materials and methods

2.1. Experimental animals

A total of 12 SPF *L. vannamei* samples including adult shrimp and larvae were randomly collected from prime division of Tamil Nadu aquaculture environment for the study. The shrimps used in culture were imported from Brazil's aquaculture grounds through CP Aqua India Pvt, Ltd and other leading aquaculture industries. The samples collected were kept in icebox and taken to lab. Collected samples were stored at −20 ℃ for further use. DNA was isolated from gills, appendages, and pleopod legs, etc., from adult shrimp and larvae.

2.2. Identification of WSSV free L. vannamei farms

The WSSV non-infected *L. vannamei* shrimp farms were located in seven regions. It is located in Velankanni, Agaram, Poobukar, Karapedakai, Puthupattinam, Mahendra Palli and Thirukarkavur. It is a modified extensive and semi-intensive system culturing *L. vannamei* with CAA regulatory. The farm area is about 12 − 360 hectares having ten ponds each with I ha area. They were used in creek water as well as bore water. The water quality parameters like dissolved oxygen, temperature and salinity were 6.2-7.5 ppm, 23-25 ℃ and 15-43 ppt respectively. A total of 12 samples SPF *L. vannamei*, adult shrimp and larvae were randomly collected from the above-mentioned regions.

2.3. Identification WSSV outbreak L. vannamei farms

The WSSV infected *L. vannamei* shrimp farms were located in five regions. It is located in Nagoore, Nagapattinam, Paravai, Sirkali and Kalpakkam. It is a modified extensive and semi-intensive system culturing *L. vannamei* with non-coastal aquaculture authority regulatory. The farm area is about 7-120 hectares having ten ponds each with 0.8-I ha area. They were used in Creek water as well as bore water. The water quality parameters like dissolved oxygen, temperature and salinity were 6.5 − 7.5 ppm, 24 ℃ − 25 ℃ and 20 − 41 ppt respectively. A total of 12 samples SPF *L. vannamei*, adult shrimp and larvae were randomly collected from the above-mentioned region.

2.4. Sample preservation

The tissue sample for DNA extraction is cut into small pieces (< 5-7 mm) to permit adequate fluid penetration and preserved in invigorated 95% ethanol using 1.5 mL label tubes. The tubes were then stored under refrigerated conditions and it was replaced with fresh 95% ethanol after few days of preservation to optimize DNA preservation.

2.5. Isolation of genomic DNA from L. vannamei

About 300 μL of TEN was added to the tube and homogenized and then centrifuged at 3 000 rpm for 10 min. Supernatant was collected in a fresh tube and 1% SDS was added and mixed well. Proteinase K to a final concentration of 100 μg/mL was added, mixed well by inverting and incubated at 55 ℃ for 1 h in water bath. Equal volume of tris saturated phenol: chloroform: isoamylalcohol (25:24:1) was added and mixed by inverting followed by centrifugation at 12 000 rpm for 10 min. The upper aqueous layer (containing DNA) obtained after centrifugation was collected using wide mouthed pipette tip. The aqueous layer was once again extracted with equal volume of chloroform: isoamyl alcohol (24:1) and centrifuged at 1 000 rpm for 10 min. To the aqueous phase 1/10 volume of 3 mol sodium acetate (pH 5.2) was added and mixed by inverting. And then qual volume of absolute alcohol was added and mixed gently. The DNA formed a visible precipitate. The DNA was pelleted by centrifugation at 12 000 rpm for 10 min. The pellet was washed twice with 70% ethanol. After air drying (to remove traces of ethanol) DNA was dissolved in 100 μL of TE buffer (10 mmol Tris, 1 mmol EDTA, pH=8).

2.6. Virus purification

Viral DNA was isolated from purified virions by treatment with proteinase K (0.2 mg/mL) and sarkosyl (1%) at 65 ℃ for 2

h, followed by phenol and chloroform extraction and dialysis against TE. The purity and concentration of the DNA were determined by agarose gel electrophoresis.

2.7. PCR analysis

WSSV-DNA was detected using a commercial 2-step PCR detection kit. The PCR was performed using the method of 2-step WSSV diagnostic nested PCR, described by IQ2000 Farming IntelliGene Tech. Corp, Taipei, Taiwan. Electrophoresis was executed by loading 12 μL of the amplified product and 5 μL DNA molecular markers onto 1.5% agarose gel with 1× TBE (Trizma, boric acid, EDTA) buffer. The gel was stained using ethidium bromide solution (1 μg/mL) for 30 min, and the bands were visualised by UV transillumination and GelDoc system. Accomplished WSSV negative and positive results were interpreted with the help of performed gel, under UV exposure GelDoc System.

2.8. Histopathology

For histological consequence, *L. vannamei* organ tissues were collected from WSSV infected experimental tanks (three from each tanks). *L. vannamei* were dissected and segregated the following organs viz., gills, lymphoid organ, haematopoietic tissue, and stomach, mid and hind gut. The dissected organs was immediately fixed in Davidson's fixative for histology, and the rest was fixed in 95% ethanol for PCR. For histology study, routine procedures were followed for preparation, sectioning, and staining with haematoxylin and eosin. The polychaete organs from the experimental and control shrimps were examined histologically for WSSV-specific manifestations following the routine diagnostic protocol of Lightner[18].

3. Results

The results were considered to have the risks of introduction of exotic pathogens, ecological risks of escape and establishment, environmental risks of intensive culture, economic risks of competition in marketing and social risks of small farmers, the study identified high risk scenarios for the import and culture of *L. vannamei*.

3.1. Gross pathology of WSSV in outbreak region

Grossly visible white spots were usually rounded and consisted of a peripheral whitish-brown ring enclosing a brownish central area demarcated by small cavities assembled in bead-like rows. Numerous scattered melanised spots and cavities were found in the central area. White spots first appeared on the carapace and on the fifth-sixth abdominal segments, and later on the shell of the whole body. Sizes of the spots varied from barely visible dots to spots of 3 mm in diameter. The initial microscopic spots mainly appeared as separate tiny dots but they were sometimes also arranged in bead-like order.

Table 1
PCR result of WSSV in source water samples collected from Tamil Nadu aquaculture region.

Infection range / Viral copies / Base pair	Source of sample	Nested PCR result
Low / 20 / 296 bp	Nagoore	positive
Severe / 2000 / 910, 650, 296 bp	Nagapattinam	positive
Severe / 2000 / 910, 650, 296 bp	Paravai	positive
Low / 20 / 296 bp	Sirkali	positive
Severe / 2000 / 650, 296 bp	Kalpakkam	positive
Negative internal control band / 848 bp	Velankanni	Negative
Negative internal control band / 848 bp	Agaram	Negative
Negative internal control band / 848 bp	Poobukar	Negative
Negative internal control band / 848 bp	Karapedakai	Negative
Negative internal control band / 848 bp	Puthupattinam	Negative
Negative internal control band / 848 bp	Mahendra Palli	Negative
Negative internal control band / 848 bp	Thirukarkavur	Negative

Table 2
PCR result of WSSV shrimp L. vannamei samples collected from Tamil Nadu aquaculture region.

Infection range / Viral copies / Base pair	Source of sample	Nested PCR Result
Low / 20 / 296 bp	Nagoore	positive
Severe / 2000 / 910, 650, 296 bp	Nagapattinam	positive
Severe / 2000 / 910, 650, 296 bp	Paravai	positive
Severe / 2000 / 910, 650, 296 bp	Sirkali	positive
Moderate / 200 / 650, 296 bp	Kalpakkam	positive
Negative internal control band / 848 bp	Velankanni	Negative
Negative internal control band / 848 bp	Agaram	Negative
Negative internal control band / 848 bp	Poobukar	Negative
Negative internal control band / 848 bp	Karapedakai	Negative
Negative internal control band / 848 bp	Puthupattinam	Negative
Negative internal control band / 848 bp	Mahendra Palli	Negative
Negative internal control band / 848 bp	Thirukarkavur	Negative

The spots appeared yellowish-brown and opaque under the microscope rather than white as seen by the naked eye. They were mainly embedded in the cuticle but some portions extended to its inner surface. Large, whitish patches visible to the naked eye also occurred when the spots enlarged and coalesced, resulting in an overall whitish discoloration of the shell.

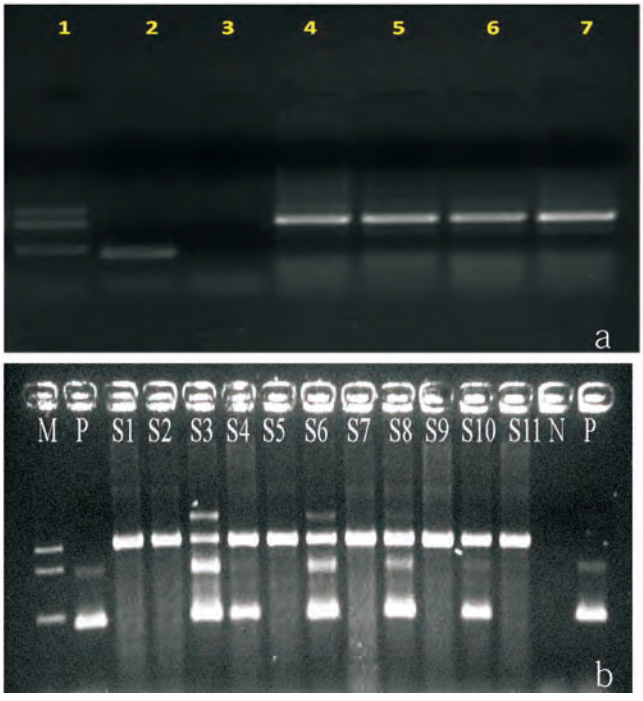

Figure 1. Photograph of agarose gel electrophoresis of PCR product of the source water samples obtained from WSSV contaminated and non-contaminated SPF *L.vannamei* creeks, estuary & back water. a) Non-contaminated source water: Lane 1- Molecular weight marker (848,650,333 bp), Lane 2- Positive control (910, 650 and 296 bp), Lane 3- Negative control (yeast tRNA), Lane 4- WSSV-ve sample (848 bp) (Sample 6), Lane 5- WSSV-ve sample (848 bp) (composite samples- sample 7 and 8), Lane 6- WSSV-ve sample (848 bp) (composite sample 9 and 10), Lane 7- WSSV-ve sample (848bp) (composite sample 11 and 12); b)WSSV contaminated source water samples: Lane 1- Molecular weight marker (848, 650, 333bp), Lane 2- Positive control (910, 650 and 296 bp), Lane 3, 4- WSSV-ve water sample 6 and 7 (848 bp), Lane 5, 8- WSSV severe +ve sample - 910, 650, 296 bp (sample 2 and 3), Lane 6,12- WSSV low, moderate +ve sample - 650, 296 bp (sample 1-4), Lane 7, 9, 11, 13- WSSV-ve (848 bp) (sample 8-11), Lane 13- WSSV severe level +ve sample- 910, 650, 296 bp (sample 5), Lane 14 and 15- Negative control (yeast tRNA) and Mod. Positive control (650 and 296 bp).

3.2. Nested PCR detection of WSSV

The results of PCR analysis on different organs obtained from different WSSV infected region of Tamil Nadu, aquaculture environment showed the appearance of a prominent band from the PCR amplified product of WSSV-DNA at internal control band 848 bp at non-infected areas. Although low infection positive bands (20 copies) were shown at 296 bp continued from initial stage of the infection region. The moderate and severe levels were observed as 650 bp and 910 bp (200 and 2 000 copies), during the severe out break periods. The product band ranging between 296 to 910 bp was found during the entire period from the WSSV outbreak region of SPF *L.vannamei* shrimps, which exposed to WSSV through native shrimp of *P. monodon*. The water samples' results were presented in low level range at sample 1 and 4 (Table 1). According to above specified consequence, WSSV impact was not much different between source water and culture animal. The WSSV positive was observed in 5 sample locations from the total number (12) of samples (Table 1 and 2). Similar WSSV positive level was recorded in both the groups of samples. The infection range was interrelated between the water and shrimp samples. The transmission of WSSV pathogen was passing on through source water, which was connected in the native species culture farm drainage canal. The WSSV water pollutions were started from native species culture environment and transmitted through the SPF *L. vannamei* shrimp farm environment. Due to lack of anticipation control capacity and unaware about CAA regulations, the WSSV water pollutions were contaminated in the particular water source of creeks, estuary and backwater during the period of WSSV outbreak.

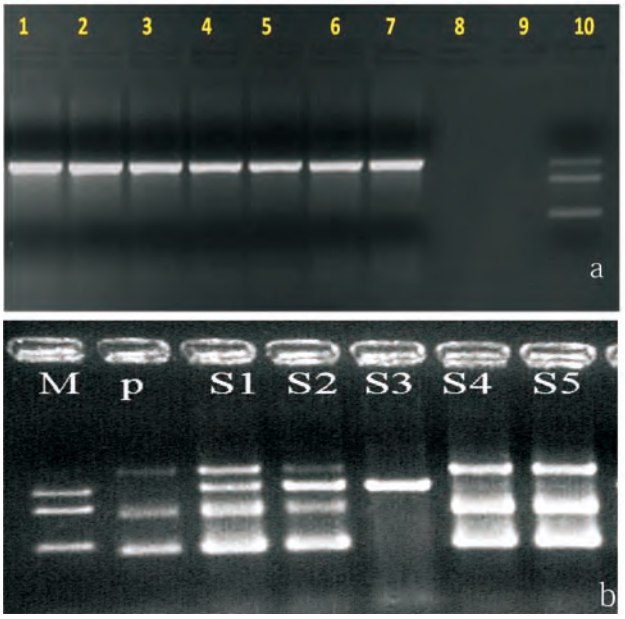

Figure 2. Photograph of agarose gel electrophoresis of PCR product of the *L.vannamei* shrimps obtained from WSSV affected and non-affected SPF *L.vannamei* shrimp farm. a) Non-affected shrimp farm: Lane 1- WSSV-ve sample (848 bp, sample 6), Lane 2- WSSV-ve sample (848 bp, sample 7), Lane 3,4 WSSV-ve sample (848 bp, samples 8 and 9), Lane 5, 6- WSSV-ve sample (848 bp, sample 10 and 11), Lane 7- WSSV-ve sample (848 bp, sample 12), Lane 8,9- Negative control (yeast tRNA) Primer control (IQ2000 primer), Lane 10- Positive control (910, 650 and 296 bp); b) WSSV affected shrimp samples: Lane 1- Molecular weight marker (848, 650, 333bp), Lane 2- Positive control (910, 650 and 296 bp), Lane 3- WSSV severe +ve sample (910, 650, 296 bp, sample 2), Lane 4- WSSV severe +ve sample (910, 650, 296 bp, sample 3), Lane 5- WSSV low +ve sample (296 bp, sample 1) , Lane 6- WSSV severe +ve sample(910, 650, 296 bp, sample 4), Lane 7- WSSV severe level +ve sample (910, 650, 296 bp, sample 5).

3.3. Observation of WSSV contaminated water in L. vannamei culture premises region

In India, shrimp aquaculture is being practiced mostly along the brackish water creeks and canals in clusters of farms drawing and draining water from the same source. There are around 100 000 small-scale shrimp farmers

cupying about 140 000 hectares with an annual production of about 140 000 tonnes[1]. Indian shrimp culture has passed through three distinct phases. Out of the 12 creeks, backwater and estuary water samples (water was drawn from WSSV outbreak farms) analyzed only 5 were found to be positive for WSSV by nested PCR. Positive samples were further used for studying the deletion, variable and transposes region of native species *P. monodon* and non-native species SPF *L. vannamei*. The viral content was noticed with severe infection (2 000 copies/ 910, 650 and 296 bp) in sample 3 and 2 of the backwaters. In low level of WSSV infection were recorded in sample 1 and 4 (20 copies/ 296 bp) respectively (Figure 1a and b).

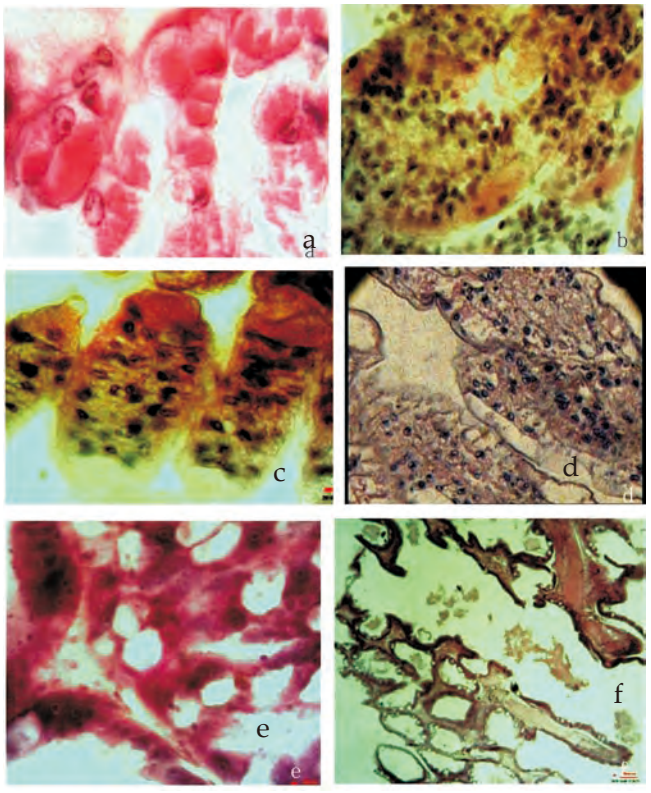

Figure 3. Histological observation of severely WSSV infected L. vannamei shrimps.
a) Hematopoietic tissue with basophilic intranuclear inclusion bodies characteristic of white spot syndrome virus (WSSV) in L. vannamei (H and E. 100 X); b) Intranuclear inclusion bodies characteristic of WSSV infection in the hind gut region of L. vannamei showing signs of WSSV (H and E 40 X); c) Intranuclear inclusion bodies characteristic of WSSV infection in the gill Lamella cells of L. vannamei showing of WSSV (H and E 100 X); d) Intranuclear inclusion bodies characteristic of WSSV infection in the hind gut region cells of L. vannamei showing signs of WSSV (H and E 100 X); e) Intranuclear inclusion bodies characteristic of WSSV infection in the vacuolization of Hepatopancreatic tissue in L. vannamei showing of WSSV (H and E 100 X); f) Hepatopancrese tissue degeneration characteristic of WSSV infection in the Hepatopancreatic tissue in L. vannamei showing of WSSV (H and E 4 X).

3.4. Environmental effects of WSSV contaminated water

L. vannamei is tolerant of a wide range of salinities, especially very low salinity. This means that it is currently cultured in both inland and coastal areas. Just as with the farming of other Penaeid species, this raises a number of potential environmental issues. Environmental concerns for *L. vannamei* culture include potential impacts on: (1) natural and agricultural habitats, caused by poorly sited or managed shrimp farms; and (2) effects of farm effluents on water quality in inland and coastal areas. Although there are differences in the locations where *L. vannamei* and native Penaeid species are farmed, there are likely to be no major differences in the impacts on habitats. In Tamil Nadu, *L. vannamei* is commonly farmed in shrimp farms that have previously produced *P. monodon*. Therefore, no significant new impacts on the habitats of coastal or agriculture areas are anticipated. Although there has been some expansion of *L. vannamei* into new farming areas, impact such as farming on the surrounding natural environment is not considered significant, provided adequate measures are taken. As in the case of *P. monodon*, particular care is essential when culturing *L. vannamei* in areas with seasonal estuary. Normal sitting practices and good farm management for reducing impacts on surrounding habitats should be followed. Where farms practice limited water exchange, recycling of pond water or use of effluent treatment, then impacts on the surrounding environment can be reduced or eliminated. The trend in farming of *L. vannamei* in Tamil Nadu and the Andhra Pradesh is towards the use of limited water exchange and closed or semi-closed farming systems, i.e., fenced and netted entire farm premises, avoided dissimilar species culture in same *L. vannamei* grounds and maintained reservoir, thus the impacts on the environment are less.

3.5. Observation of WSSV infected tissues L. vannamei in the infected and non infected regions

Sample 1 recorded low level of the positive results compared to sample 2, 3 and 5. In particular, the WSSV outbreak period showed the entire five locations sample (in both groups) were presented positive bands, which was landed at 296-910 bp in 20-2 000 copies. (Figure 2a and b). The sample 6-10 locations were presented the internal control band, which showed 848 bp. The results revealed that the sample of the majority of locations did not show any WSSV infection, because of CAA regulatory farms. They were followed CAA regulation, and also they made the fenced structure, maintained reservoir, cultured in single species in one farms and netted in entire farm regions. In addition non-regulatory shrimp farms were affected the WSSV pathogenicity and were found in severe level (910, 650, 296 bp and 2 000 viral load copies).

3.5. Histopathological observation of WSSV

Histological observation of severely WSSV infected *L. vannamei* shrimps revealed degenerated cells characterized by intranuclear inclusions in the tissues of WSSV infected mid-gut gland, lymphoid organ, gill lamellae, gut epithelium. Hematopoietic tissues with basophilic intranuclear inclusion bodies characteristic were observed in moderate infection of WSSV in *L.vannamei* (Figure 3a). Intranuclear inclusion bodies characteristic of WSSV infection in the hind gut region of *L. vannamei* showing signs of WSSV is shown in Figure 3b. Intranuclear inclusion bodies characteristic of WSSV infection in the gill lamella cells of *L. vannamei* is shown in Figure 3c. WSSV infection in

the hind gut region is shown in Figure 3d. Figure 3e depicts the intranuclear inclusion bodies of WSSV infection in the vacuolization of hepatopancreatic tissue in *L. vannamei*. The hepatopancreatic tissue degeneration characteristic of WSSV infection in *L. vannamei* is shown in Figure 3f.

4. Discussion

In the present study, the cumulative WSSV mortalities of SPF *L. vannamei* were obtained from the pathogenicity outbreak of aquaculture environment. The current investigation showed that the WSSV present to infected from low level to severe level. The PCR findings revealed that the SPF Pacific white shrimp *L. vannamei* was presented around five places of Tamil Nadu, the different ranges of WSSV viral infections by the 20-2 000 copies. The rests of region sample were recorded as a negative towards WSSV pathogen; it was shown at 848 bp as an internal control band. The viral DNA was presented the low levels (296 bp/20 copies) in sample 1 and moderate level (650, 296 bp/200 copies) from the sample 2 respectively. Albeit, WSSV severe level infection (910, 650, 296 bp/2000 copies) were observed from the three places of Tamil Nadu, most of WSSV infection were resulted from native shrimp *P. monodon*. PCR results also confirmed the above surveillance from the majority of places. Similarly, the distribution of WSSV in the distinctive place's samples of brood stock examined; Three were apparently healthy while 7 showed gross signs of WSSV infection. The distribution of WSSV in different organs and tissues of *L.vannamei* was diagnosed using histopathological tools. The histopathological results showed different WSSV appearances in different tissue samples. The severe infected moribund shrimps strongly implied the presence of infectious virus in all these tissues and organs of *L. vannamei*. The intranuclear inclusion bodies characteristic of WSSV infection in the gill lamella cells of *L. vannamei* showed WSSV in sample 3 and 4. Hematopoietic tissues with basophilic intranuclear inclusion bodies characteristic of WSSV in *L. vannamei* from sample 1 and 5. The present study showed a high frequency of WSSV by PCR in captured *L. vannamei* samples. WSSV prevalence was also reported to be quite high in other SPF *L. vannamei* animal in native species *P. monodon* through water contamination, collected in nearby *P. monodon* shrimp farms.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Yan DC, Dong SL, Huang J, Zhang JS. White spot syndrome virus (WSSV) transmission from rotifer inoculum to crayfish. *J Invertebr Pathol* 2007; **94**: 144-148.

[2] Shi Z, Wang H, Zhang J, Xie Y, Li L, Chen X, et al. Response of crayfish, *Procambarus clarkii*, haemocytes infected by white spot syndrome virus. *J Fish Dis* 2005; **28**: 151-156.

[3] Zeng Y, Lu CP. Identification of differentially expressed genes in haemocytes of the crayfish (*Procambarus clarkii*) infected with white spot syndrome virus by suppression subtractive hybridization and cDNA microarrays. *Fish Shellfish Immunol* 2009; **26**: 646-650.

[4] Badhul Haq MA, Nazar AR, Asraf Ali B, Somnath C, Shalini R. Assessment of *Artemia franciscana* as a probable vector for WSSV transmission to *Macrobrachium idella idella* (Hilgendorf 1898). *Int J Chem Anal Sci* 2011; **2**(9): 1159-1170.

[5] Pongsomboon S, Tang S, Boonda S, Aoki T, Hirono I, Yasuike M, et al. Differentially expressed genes in *Penaeus monodon* hemocytes following infection with yellow head virus. *BMP Report* 2008; **41**: 670-677.

[6] Syed Musthaq S, Madhan S, Sahul Hameed AS, Kwang J. Localization of VP28 on the baculovirus envelope and its immunogenicity against white spot syndrome virus in *Penaeus monodon*. *Virol* 2009; **391**: 315-324.

[7] Wang XW, Xu WT, Zhang XW, Zhao XF, Yu XQ, Wang JX. A C-type lectin is involved in the innate immune response of Chinese white shrimp. *Fish Shellfish Immunol* 2009; **27**: 556-562.

[8] Escobedo-Bonilla CM, Alday-Sanz V, Wille M, Sorgeloos P, Pensaert MB, Nauwynck HJ. A review on the morphology, molecular characterization, morphogenesis and pathogenesis of white spot syndrome virus. *J Fish Dis* 2008; **31**: 1-18.

[9] Li HX, Meng XL, Xu JP, Lu W, Wang J. Protection of crayfish, *Cambarus clarkii*, from white spot syndrome virus by polyclonal antibodies against a viral envelope fusion protein. *J Fish Dis* 2005; **28**: 285-291.

[10] Rameshthangam P, Ramasamy P. Antiviral activity of bis (2-methylheptyl) phthalate isolated from *Pongamia pinnata* leaves against white spot syndrome virus of *Penaeus monodon fabricius*. *Virus Res* 2007; **126**: 38-44.

[11] Kim DK, Jang IK, Seo HC, Shin SO, Yang SY, Kim JW. Shrimp protected from WSSV disease by treatment with egg yolk antibodies (IgY) against a truncated fusion protein derived from WSSV. *Aquaculture* 2004; **237**: 21-30.

[12] Natividad KDT, Hagio M, Tanaka M, Nomura N, Matsumura M. White spot syndrome virus (WSSV) inactivation in *Penaeus japonicus* using purified monoclonal antibody targeting viral envelope protein. *Aquaculture* 2007; **269**: 54-62.

[13] Kim CS, Kosuke Z, Nam YK, Kim SK, Kim KH. Protection of shrimp (*Penaeus chinensis*) against white spot syndrome virus (WSSV) challenge by double-stranded RNA. *Fish Shellfish Immunol* 2007; **23**: 242-246.

[14] Xu JY, Han F, Zhang XB. Silencing shrimp white spot syndrome virus (WSSV) genes by siRNA. *Antiviral Res* 2007; **73**: 126-131.

[15] Vignesh R, Karthikeyan BS, Periyasamy N, Devanathan K. Antibiotics in aquaculture: An overview. *South Asian J Exp Biol* 2011; **1**(3): 114-120.

[16] Badhul Haq MA, Kathiresan K. Marine viral diversity. [dissertation] UNU-INWEH-UNESCO: International training course on coastal biodiversity in mangroves ecosytems; 2010 tober 1-15, p. 268-282.

[17] Badhul Haq MA, Kavitha N, Vignesh R, Shalini R. Identification and sequence based detection of WSSV infecting SPF Litopeneaus vannamei (Boone, 1931) in captivity. *Int J Pharm Bio Sci* 2012; **3**(1): B547-559.

[18] Lightner DV. *A handbook of shrimp pathology and diagnostic procedures for diseases of cultured penaeid shrimp*. LA: World Aquaculture Society; 1996, p. 304.