International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 27-05-2025, Accepted: 26-06-2025, Published: 11-07-2025
Volume 10, Issue 07, 2025, Page No. 84-89

Prevalence of White Spot Syndrome Virus (WSSV) in wild-caught Tiger Shrimp (Penaeus monodon)

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Abstract
White Spot Syndrome Virus (WSSV) is a major viral pathogen affecting penaeid shrimp, causing substantial economic losses in aquaculture. This study investigated the prevalence of WSSV in wild-caught Penaeus monodon from three marine landing centers—Annankoil, Mudasalodai, and Samiyarpettai—along the Cuddalore coast, Tamil Nadu, India. A total of 41 shrimp samples were collected and examined through gross clinical signs, histopathological analysis, and PCR confirmation. Characteristic white spots on the carapace, reddish appendages, and loose cuticle were observed in gross examination, while histology revealed nuclear hypertrophy and eosinophilic to basophilic intranuclear inclusion bodies in gill tissues—hallmarks of WSSV infection. Molecular diagnosis using nested PCR confirmed the presence of WSSV with 941 bp amplicons. Results showed a significantly higher prevalence of WSSV in female shrimp (9.46%) compared to males (5.62%), with peak infections during February and March. The findings suggest seasonal variation in infection intensity and highlight the potential risk posed by infected wild broodstock, particularly from areas near shrimp farms. This underscores the urgent need for routine screening and adoption of specific pathogen-free (SPF) broodstock to mitigate the spread of WSSV in aquaculture systems.

Keywords: Penaeus monodon; white spot syndrome virus (WSSV); molecular diagnosis; cuddalore coast; histopathology

Introduction

Shrimp aquaculture is a billion-dollar industry that significantly contributes to the economies of several countries, particularly in Asia and South America. The rapid expansion of shrimp farming has fueled economic growth; however, it has also increased the risk of disease outbreaks, leading to considerable economic setbacks and slower development of the industry (Flegel, 2006) [14]. In aquaculture, disease is defined as any impairment that disrupts or modifies normal physiological functions. This can result from environmental stressors such as pollutants and climate change, nutritional imbalances, infectious agents, or genetic defects (Hedrick, 1998). Among these, viral pathogens pose the greatest threat to shrimp health, accounting for approximately 60% of disease-associated losses in shrimp farming, whereas bacterial pathogens contribute around 20%, with fungal and parasitic agents playing a lesser role (Flegel, 2006, 2012) [14]. It is estimated that pathogens cause up to a 40% loss in potential shrimp production in tropical regions (Lundin, 1996). The incidence and severity of infectious diseases in aquatic organisms have gained increasing attention due to climate change and intensified anthropogenic activities, which contribute to the emergence and spread of pathogens. Common disease-causing agents in shrimp include bacteria, viruses, fungi, protozoans, parasitic crustaceans, helminths, and other worms. Their impact often depends on the biological and biochemical properties of the pathogen and the host's susceptibility. In India, aquaculture is an important sector that plays a critical role in national food security. Shrimp aquaculture is the fastest-growing component of this sector, with production primarily dominated by two species: the black tiger shrimp (Penaeus monodon) and the Pacific white shrimp (Litopenaeus vannamei). Despite its rapid growth, the shrimp industry suffers major economic losses due to outbreaks of both emerging and existing diseases, particularly viral infections, which have become the leading cause of mortality in penaeid shrimp. One of the most devastating viral diseases affecting shrimp is White Spot Disease (WSD), caused by the White Spot Syndrome Virus (WSSV). WSSV is a double-stranded DNA virus belonging to the family Nimaviridae, genus Whispovirus (Mayo, 2002). It is highly pathogenic and leads to rapid mortality and severe economic loss (Flegel, 1997) [13]. The virus was first identified in 1992 in Penaeus japonicus in Taiwan (Chou *et al*., 1995) [5], and soon after in Japan (Nakano *et al*., 1994) [33]. The virions are large (70–150 nm × 250–380 nm), and the viral genome is approximately 305 kb in length (Durand *et al*., 1997; Yang *et al*., 2001) [9]. Clinically, WSSV-infected shrimp exhibit characteristic white spots (up to 3 mm in diameter) on the carapace. Other signs include reddish discoloration of the body and appendages, decreased feeding, lethargy, loose cuticle, fluid accumulation in the branchiostegites, and swollen or discolored hepatopancreas (Inouye *et al*., 1994; Wang *et al*., 1999; Nadala *et al*., 1998; Karunasagar & Karunasagar, 1999; Sahul Hameed *et al*., 1998) [20]. While the presence of white spots is a classic indicator of infection, disease severity increases with signs such as color change to reddish-brown, surface aggregation, and rapid drop in feed intake—often preceding mass mortality within hours or days. WSSV transmission occurs both vertically (from infected broodstock to offspring) and horizontally (via ingestion of infected tissue or exposure to contaminated water). Healthy-appearing animals and even dead shrimp can act as carriers (Lo *et al*., 1997; Chou *et al*., 1998). The virus targets tissues of ectodermal and mesodermal origin, including the cuticular epithelium and subcuticular connective tissues. Due to its wide host range, WSSV can infect nearly all cultured shrimp species, including *P*. monodon, L. vannamei, L. stylirostris, F. chinensis, and even freshwater species such as Macrobrachium rosenbergii.

It is thus classified as a Category C-1 pathogen (Lotz, 1997). In India, WSSV was first reported in 1994 on the east coast (Mohan *et al*., 1998) [31] and rapidly became endemic across all shrimp farming regions, contributing to cyclic epidemics. The primary sources of infection include infected post-larvae (PL), broodstock, and frozen shrimp (Shankar & Mohan, 1998) [31]. The shrimp-farming industry now emphasizes prevention and early detection. Polymerase Chain Reaction (PCR) is widely recommended for screening PL and broodstock (Limsuwan, 1997; Lo & Kou, 1998). However, PCR alone cannot differentiate between contamination and active infection. Confirmatory diagnosis using histology, in situ hybridization, immunohistochemistry, or electron microscopy is essential (Stentiford *et al*., 2009). Histopathological signs of WSSV infection typically include hypertrophied nuclei and eosinophilic to basophilic intranuclear inclusion bodies in target tissues. This study aims to explore the prevalence of WSSV in wild-caught *Penaeus monodon*, with a focus on molecular (PCR) and histological confirmation to better understand the virus's presence in natural shrimp populations.

Materials and Methods

1. Sample Collection

Black tiger shrimp (*Penaeus monodon*) exhibiting suspected signs of WSSV infection were collected from the Chidambaram fish market. The samples were transported to the laboratory in an ice-cold container to maintain tissue integrity and subsequently stored at −20 °C until further analysis.

2. Gross Observation and Wet Mount Study

All shrimp were subjected to external examination to assess gross pathological signs of WSSV infection. Healthy shrimp exhibited a glassy white coloration with intact appendages (antennae, eyes, pleopods, pereiopods). In contrast, infected individuals showed visible white spots on the carapace and abdomen. These spots were generally circular, sometimes ringed, and varied in size from barely visible up to 3 mm in diameter. The opacity of the carapace, particularly in the cephalothorax region, was another hallmark of suspected WSSV infection. For microscopic evaluation, carapace tissue was mounted on a clean glass slide with a drop of saline, covered with a coverslip, and observed under a compound light microscope at varying magnifications to identify tissue-level abnormalities indicative of WSSV.

3. DNA Isolation

Gill tissues from the shrimp were dissected and preserved in 95% ethanol. Genomic DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen) following the manufacturer's protocol. The integrity and quality of isolated DNA were verified by electrophoresis on a 1% agarose gel stained with ethidium bromide and visualized using a Gel Documentation System (Eppendorf).

4. Polymerase Chain Reaction (PCR) Amplification

The extracted DNA samples were screened for WSSV using a two-step nested PCR approach targeting WSSV-specific gene sequences.

First-
Step PCR: Primers: (146F1: 5' ACTACTAACTTCAGCC TATCTAG-
3'; 146R1: 5' TAATGCGGGTGTAATGTTCTTACG-3'). PCR was performed for 40 cycles. 5 µL of the product was electrophoresed on a 1.5% agarose gel to verify amplification.

Second-Step PCR: Primers: (146F2: 5'-GTAACTGCCCCTTCCATCTCCAA-3'; 146R2: 5'-TACGGCAGCTGCTGCACCTTGT-3'). 10 µL of the first-step product was used as a template for nested PCR. PCR products were again resolved on a 1.5% agarose gel and visualized under UV light. The expected band size for a WSSV-positive sample was 941 bp. Positive and negative controls were included in every PCR run to validate the results.

5. Histological Investigation

Both healthy and suspected WSSV-infected tissues were subjected to histopathological analysis.

Fixation: Tissue samples were fixed in Davidson's fixative at room temperature for 48 hours and subsequently transferred to 70% ethanol.

Processing and Embedding: Fixed tissues were dehydrated in graded ethanol series, cleared in xylene, and embedded in paraffin wax.

Sectioning and Staining: Sections of 5 µm thickness were prepared using a rotary microtome and stained with hematoxylin and eosin (H&E).

Observation: Prepared slides were mounted with DPX (distyrene-plasticizer-xylene) and examined under a compound light microscope for characteristic histological changes indicative of WSSV infection, such as hypertrophied nuclei and intranuclear inclusion bodies.

Results

Sample collection

Samples of *Penaeus monodon* were collected from three prominent marine landing centers along the Cuddalore coast of Tamil Nadu, India. These included the Annankoil Landing Center (11.3565° N, 79.7542° E), Mudasalodai Landing Center (11.4498° N, 79.7675° E), and Samiyarpettai Landing Center (11.4395° N, 79.7732° E). These locations were selected based on their high shrimp landings and proximity to estuarine and marine environments known for natural shrimp populations. The geographical coordinates of each site were recorded to accurately map the sampling area and facilitate further ecological correlation with WSSV prevalence.

Sample Identification

All collected specimens were morphologically identified as Penaeus monodon based on FAO taxonomic keys (Fabricius, 1798). The identification was based on characteristic features such as body coloration, banded abdominal segments, and distinct pereiopods.

Gross Observation

Out of the 41-shrimp collected from the Chidambaram fish market, a majority showed visible signs suggestive of WSSV infection. Characteristic white spots of varying diameters (ranging from <1 mm to 3 mm) were observed on the carapace and abdomen of suspected individuals. The carapace appeared opaque, and the body coloration was abnormal in many cases. Other gross signs included reddish discoloration of appendages, reduced responsiveness, and loose cuticles in some samples.

Fig 1: Carapace of infected and healthy shrimp

Fig 2: Panaeus monodon – a. Healthy Shrimp; b. Infected shrimp.

a. Carapace with white spot: b. Normal healthy shrimp's carapace.

Microscopic Observation

Histological examination of the gill tissue from *Penaeus monodon* revealed distinct pathological changes characteristic of White Spot Syndrome Virus (WSSV) infection. Under light microscopy (H&E staining), transverse sections of the gill filaments showed significant nuclear hypertrophy in epithelial cells, with clearly visible intranuclear inclusion bodies—a hallmark feature of WSSV. These inclusion bodies appeared eosinophilic to basophilic and were located centrally within the enlarged nuclei, as indicated by the arrows in the micrograph. Additionally, the normal architecture of the gill tissue appeared disrupted, suggesting cytopathic effects due to active viral replication. These microscopic changes further confirm the presence and pathological impact of WSSV in the infected shrimp, supporting the gross and molecular diagnostic findings.

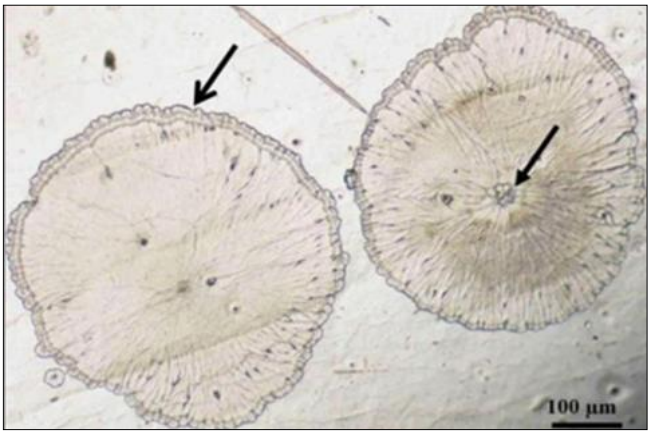

Fig 3: Microscopic view of White Spot of Panaeus monodon under the light microscope

Prevalence of WSSV

The results depicted in the bar chart illustrate the monthly variation in the number of *Penaeus monodon* shrimp observed and the corresponding incidence of White Spot Syndrome Virus (WSSV) infections in both males and females from November to March. A gradual increase in the total number of shrimps observed was noted over the months, starting from 533 in November and rising steadily to a peak of 1,625 in March. This upward trend may be attributed to seasonal abundance, improved sampling effort, or changes in shrimp migration patterns. In terms of WSSV infections, a clear pattern emerged where female shrimp consistently showed higher infection rates compared to males throughout the study period. In November, 74 infected females were recorded compared to 26 infected males. This disparity continued each month, culminating in March with 127 infected females and 94 infected males. The month of March also recorded the highest overall infection count. The progressive rise in infection numbers, particularly from January onwards, suggests an intensification of WSSV transmission during the late winter to early spring months. The higher infection rates in females may be linked to physiological differences such as larger body size, reproductive status, or behavioral factors that increase their exposure to the virus. Overall, the findings highlight a seasonal trend in WSSV prevalence, with infection rates peaking towards the end of the sampling period. These results emphasize the importance of regular monitoring, especially during the high-risk months, to better manage and mitigate the impact of WSSV on wild shrimp populations.

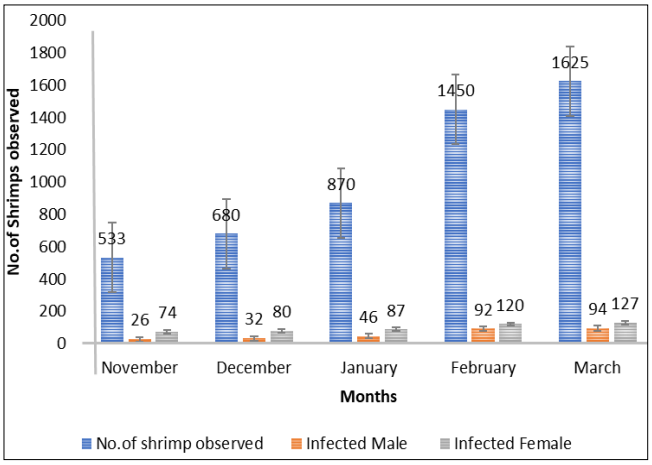

Fig 4: Monthly prevalence of WSSV infection in Penaeus monodon

DNA Isolation and PCR Confirmation of WSSV

Genomic DNA was successfully extracted from four randomly selected shrimp samples using the Qiagen DNeasy Blood and Tissue Kit. High molecular weight DNA bands were visualized on 1% agarose gel, confirming successful isolation. All four DNA samples, including a known WSSV-positive control, were subjected to nested PCR. Amplification produced distinct bands of 941 base pairs, the expected product size for WSSV, confirming infection. Negative controls showed no amplification, validating the specificity of the assay.

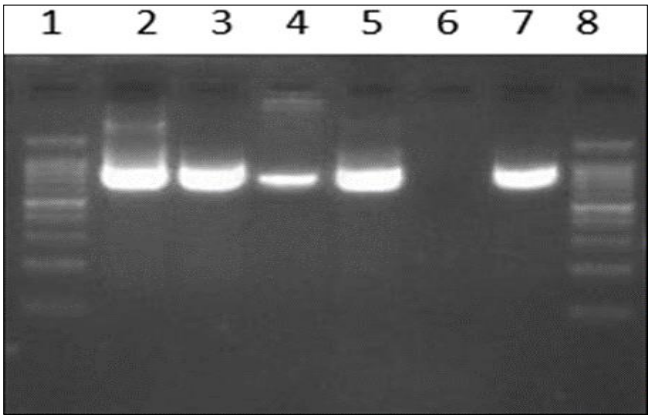

Fig 5: Gel image showing PCR diagnosis of clinically normal and WSSV-infected gill tissue.

Lane 1, 100 bp ladder; lanes 2, 3, 4 & 5, clinically infected shrimpsample; lane 6, negative control; lane 7, positive control; lane 8, 100 bp ladder

Histopathological Examination

Histological comparison of gill tissues from Penaeus monodon revealed clear differences between healthy and WSSV-infected individuals. Figure A represents the normal histoarchitecture of gill filaments, showing well-organized lamellae with intact epithelial layers and no visible cellular abnormalities. In contrast, Figure B illustrates gill tissue from a WSSV-infected shrimp, exhibiting distinct pathological changes. The lamellar structure appears disrupted, and the presence of enlarged, basophilic intranuclear inclusion bodies (indicated by blue arrows) is evident within the epithelial cells. These inclusion bodies are characteristic of WSSV infection and confirm viral replication within the tissues. The infected gills also show signs of tissue degeneration, nuclear hypertrophy, and possible hemocyte infiltration. These histopathological observations strongly support the molecular findings and provide visual confirmation of the virus-induced damage in WSSV-positive shrimp.

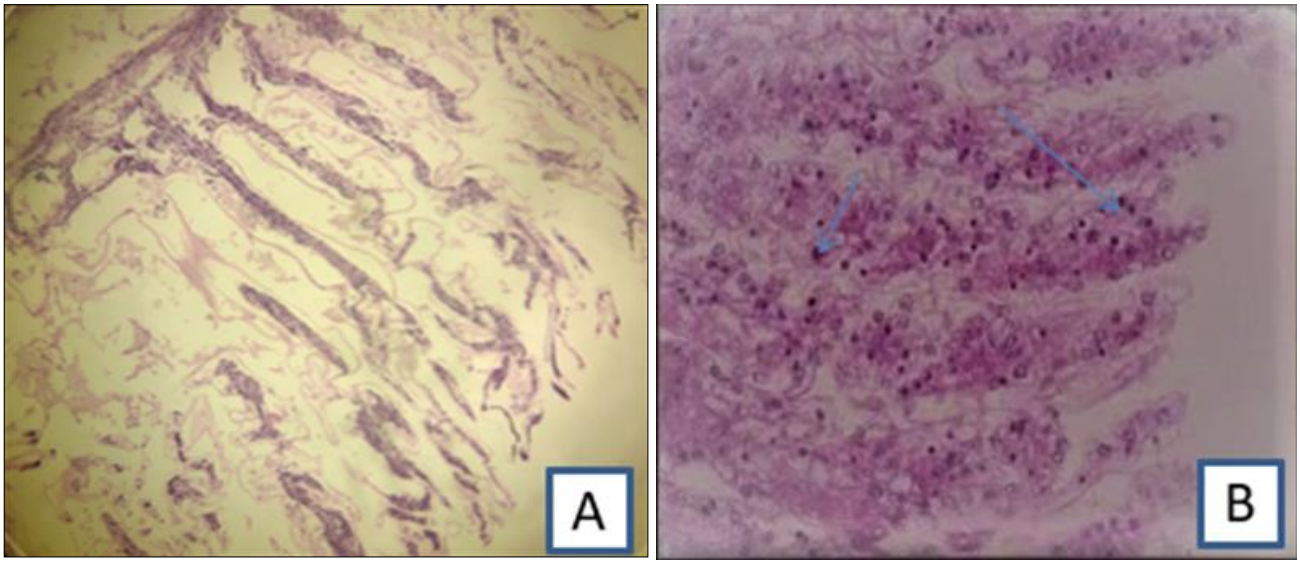

Fig. 6: A—Normal gill; B—WSSV-infected gill (basophilic inclusion bodies)

Discussion

The present study highlights a notably high prevalence of White Spot Syndrome Virus (WSSV) in wild-caught Penaeus monodon sampled from key landing centers along the Cuddalore coast, Tamil Nadu. This finding is consistent with previous reports indicating the presence of WSSV in both cultured and wild shrimp populations in India and other parts of Southeast Asia (Mohan *et al*., 1998; Flegel, 2006) [14, 31]. The observed prevalence of WSSV-positive shrimp, confirmed by gross signs, histopathological changes, and nested PCR, underlines the continuing threat that WSSV poses to shrimp health and coastal aquaculture. Gross signs of infection, including white spots on the carapace and discolored appendages, were present in a large portion of the 41 samples examined. These clinical manifestations correspond with classical WSSV symptoms described in earlier studies (Lightner, 1996; Lo *et al*., 1996a). Although gross signs can be variable and sometimes non-specific, their appearance served as a reliable preliminary screening indicator, which was substantiated by further microscopic and molecular diagnostic tools. Histopathological observations of gill tissues revealed the presence of prominent basophilic intranuclear inclusion bodies and nuclear hypertrophy in epithelial cells, key histological markers of WSSV infection (Durand *et al*., 1997) [9]. The infected tissues showed cellular degeneration and architectural disruption compared to healthy samples. Similar histopathological findings have been previously documented by Wang *et al*. (1999) and Peinado-Guevara and López-Meyer (2006), who described WSSV-induced damage in the gills, stomach, and other ectodermal tissues. The temporal analysis of infection from November to March showed a progressive increase in both the number of shrimps sampled and WSSV-positive individuals. The highest prevalence was recorded in March, coinciding with seasonal environmental changes that may affect viral dynamics and shrimp susceptibility (Sivakumar *et al*., 2012). Factors such as temperature fluctuation, salinity shifts, and post-spawning immunosuppression are known to influence the outbreak potential of WSSV during specific seasons (Tendencia *et al*., 2004; Vaseeharan *et al*., 2003) [39]. Of particular interest is the consistent observation that female shrimp exhibited higher infection rates than males across all months. This may be attributed to biological

differences such as larger body mass, greater surface area for viral attachment, or higher physiological stress during reproductive phases. Similar gender-based disparities have been noted by Yub *et al*. (2008) [41], who suggested that female shrimp may be more prone to viral infections due to metabolic and hormonal factors. Molecular confirmation using nested PCR yielded 941 bp amplicons in all tested samples, further validating the presence of WSSV. The high sensitivity and specificity of nested PCR make it a gold standard for WSSV detection in both asymptomatic and symptomatic individuals (Lo *et al*., 1996b; Nunan *et al*., 1998). The absence of amplification in the negative control samples confirmed the assay's reliability, eliminating the possibility of false positives due to contamination. The detection of WSSV in wild shrimp from landing centers with direct marine and estuarine influence suggests that natural populations serve as potential reservoirs for the virus. This raises serious biosecurity concerns, especially when wild-caught broodstock are introduced into hatcheries without adequate screening. Vertical transmission of WSSV from broodstock to post-larvae has been documented (Huang *et al*., 2001), emphasizing the need for strict quarantine protocols and regular diagnostic surveillance. These findings underscore the critical need for continuous monitoring of wild shrimp populations, particularly during high-risk seasons, and highlight the importance of integrating histological, gross, and molecular diagnostics for effective disease surveillance. The presence of WSSV in wild *P. monodon* further supports the hypothesis that wild stocks contribute to the disease dynamics in aquaculture systems (Flegel, 2006; Walker and Mohan, 2009) [14, 40], making their monitoring an essential component of coastal disease management strategies.

Conclusion

The present study revealed a notable difference in WSSV prevalence between wild female and male Penaeus monodon, with infection rates significantly higher in females (9.46%) compared to males (5.62%). A clear seasonal pattern was also observed, with infection rates peaking during February and March, moderate in January, and lower in November and December. These findings suggest that environmental factors and seasonal dynamics play a significant role in the transmission and outbreak of WSSV in natural shrimp populations.

One potential source of WSSV infection in wild shrimp—particularly broodstock collected from shallow coastal waters—may be escapees or discarded individuals from nearby shrimp farms. This highlights a concerning link between aquaculture practices and disease persistence in wild ecosystems. Our results indicate that WSSV is well established in the local marine environment and has likely been circulating within wild *P.* monodon populations for some time. The higher infection rates observed in the early months of the year underscore the need for timely surveillance and intervention strategies. Given the current dependence on wild-caught broodstock—especially in regions where domesticated Specific Pathogen Resistant (SPR) *P. monodon* is not yet widely available—it is critical to adopt stringent biosecurity measures. Reducing the risk of pathogen introduction and promoting the use of disease-resistant seed stock are essential steps toward sustainable shrimp aquaculture and effective management of WSSV outbreaks.

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