Bioscene

Bioscene
Volume- 22 Number- 04
ISSN: 1539-2422 (P) 2055-1583 (O)
www.explorebioscene.com

A Study on the Prevalence and Management of White Spot Syndrome Virus (WSSV) Disease in Litopenaeusvannamei (Boone, 1931) Farms of West Godavari District, Andhra Pradesh, India

[1] K. Subhashini Devi, [2] Pamulapati Padmavathi, [3] Pappu Kirankumar
[1] Department of Zoology, Government College (A),
Rajahmundry, Andhra Pradesh, India
[2] Department of Zoology and Aquaculture, Acharya Nagarjuna University,
Nagarjuna Nagar – 522510, Andhra Pradesh, India
[3] Department of Zoology, P.R. Government College (A),
Kakinada, Andhra Pradesh, India

Abstract: White spot syndrome virus (WSSV) remains one of the most devastating viral pathogens of cultured penaeid shrimp worldwide, capable of causing near-100% mortalities within days under susceptible conditions. This research article synthesizes current knowledge on WSSV prevalence in Litopenaeusvannamei farms in West Godavari district, Andhra Pradesh, India, and critically reviews management approaches demonstrated in the literature and applied in regional practice. Using published molecular and field surveillance studies, diagnostic manuals and international standards (WOAH/OIE), and recent advances in immunostimulants, probiotics, diagnostics and vaccines, we summarize temporal and spatial patterns of infection, major drivers of outbreaks (seed quality, water exchange & environmental stressors, co-infections), and evaluate prevention and mitigation measures (biosecurity, pond management, screening, feed-based immunostimulants, and experimental vaccine approaches). Regional studies from Andhra Pradesh indicate recurring WSSV detection across coastal districts including West Godavari, with PCR-based surveys reporting variable prevalence depending on sampling frame and crop cycle (from low single-digit detection in routine screening to very high pond-level positivity during outbreaks). The WOAH diagnostic standards remain the backbone of surveillance and confirmatory testing; newer point-of-care and isothermal assays improve field responsiveness but require validation against standard PCR/ISH protocols. Integrated management — combining strict biosecurity, use of SPF/SPR seed, routine molecular screening, improved water and pond management, and targeted use of probiotics/immunostimulants — currently provides the most pragmatic approach for West Godavari farms. Promising experimental interventions (recombinant VP28 vaccines, RNAi approaches, mRNA and oral vaccine formulations) show efficacy in controlled trials but are not yet widely field-proven or commercially standardized.

Keywords: White Spot Syndrome Virus (WSSV), Litopenaeusvannamei, Shrimp aquaculture, Disease prevalence, Molecular diagnosis (PCR/qPCR), West Godavari District, Andhra Pradesh

Introduction

Shrimp aquaculture has emerged as one of the most rapidly expanding sectors of global food production, contributing substantially to seafood supply, rural employment, and export earnings (Valderramaet al., 2010, Paul &Vogl, 2013, FAO, 2020,). Among farmed shrimp species, the Pacific white shrimp Litopenaeusvannamei has become the dominant cultured species worldwide due to its fast growth, high productivity, tolerance to a wide range of salinities, and adaptability to intensive farming systems (Lightner, 2011; FAO, 2020). In India, commercial farming of L. vannamei has expanded rapidly since its official introduction in 2009, particularly along the east coast, where Andhra Pradesh accounts for the largest share of national shrimp production and exports (Muralidharan, 2015; Alavandiet al., 2019).

Despite the economic importance of shrimp farming, disease outbreaks remain the most critical constraint affecting sustainability and profitability. Among viral pathogens, White Spot Syndrome Virus (WSSV) continues to be the most devastating disease of penaeid shrimp globally. WSSV is a large double-stranded DNA virus belonging to the genus Whispovirus and family Nimaviridae, capable of causing rapid mass mortalities approaching 100% within a few days of clinical onset (Lo et al., 1996; Sánchez-Paz, 2010). Since its first emergence in Asia during the early 1990s, WSSV has spread worldwide and is now endemic in most shrimp-producing countries, including India (Lightneret al., 1996; Flegel, 2006).

In Indian shrimp farming systems, WSSV remains a persistent threat despite improvements in biosecurity, seed certification, and farm management practices. Several studies have documented the widespread occurrence of WSSV in cultured and wild crustaceans along the Indian coastline, indicating continuous environmental circulation of the virus (Ottaet al., 1999; Karunasagaret al., 1997). The virus is known to be transmitted both horizontally and vertically, with multiple pathways including infected post-larvae, carrier organisms, contaminated water, pond sediments, and farm equipment (Lightneret al., 1996; Sahul Hameed et al., 2003). Consequently, complete exclusion of WSSV from shrimp farming environments remains extremely challenging.

West Godavari district of Andhra Pradesh represents one of the most intensive shrimp farming regions in India, characterized by dense clusters of L. vannamei ponds, extensive canal networks, and close proximity between farms. The district's unique deltaic geography, abundant brackishwater resources, and favorable infrastructure have contributed to rapid expansion of shrimp aquaculture. However, these same features may also facilitate pathogen persistence and spread, particularly in areas with high farm density and shared water sources. Although farmers in West Godavari district routinely report crop failures associated with WSSV, systematic, field-based studies quantifying disease prevalence and evaluating management practices under real farming conditions remain limited.

Early detection and accurate diagnosis are essential components of WSSV disease management. Clinical signs such as white spots on the carapace, lethargy, reduced feeding, and reddish discoloration are suggestive but not definitive, as similar signs may occur under stress or in mixed infections (Sánchez-Paz, 2010). Molecular diagnostic methods, particularly polymerase chain reaction (PCR) and quantitative PCR (qPCR), are considered the gold standard for WSSV detection due to their high sensitivity and specificity (Lo et al., 1996; Tang & Lighter, 2000; WOAH, 2021). Histopathological examination of target tissues such as gills, cuticle, and hepatopancreas further provides confirmatory evidence through the identification of characteristic intranuclear inclusion bodies (Lightner, 1996; Karunasagaret al., 1997). Despite the availability of these tools, their routine application at the farm level remains inconsistent, and disease detection often occurs only after significant mortality has already begun.

Management of WSSV in shrimp farms relies largely on preventive strategies rather than therapeutic interventions, as no curative treatment exists once infection is established. Recommended approaches include use of specific pathogen-free (SPF) seed, pond preparation and disinfection, water quality management, control of vectors and carriers, and strict biosecurity measures (FAO, 2020; WOAH, 2021). In practice, however, the effectiveness of these measures varies widely among farms due to differences in farm size, resource availability, farmer awareness, and compliance with best management practices. In recent years, alternative strategies such as immunostimulants, probiotics, and novel diagnostic technologies have been explored to enhance shrimp health and disease resistance, but their field-level effectiveness against WSSV under commercial conditions remains variable (Alday-Sanzet al, 2020; Nolasco-Alzagaet al., 2025).

Given the continued economic losses attributed to WSSV in Andhra Pradesh, there is a clear need for location-specific studies that integrate disease prevalence data with farm management practices and diagnostic confirmation. Most existing studies have focused either on laboratory-based investigations or broad regional surveys, with limited emphasis on correlating WSSV occurrence with farm-level variables under routine production conditions. Understanding how disease prevalence varies across farming locations and management regimes is essential for developing practical, evidence-based recommendations tailored to local conditions.

In this context, the present study was undertaken to systematically investigate the prevalence and management of White Spot Syndrome Virus disease in Litopenaeusvannamei farms of West Godavari district, Andhra Pradesh, India. The study integrates field sampling, molecular and histopathological diagnostics, and assessment of farm management practices across major shrimp-farming mandals of the district. By generating empirical data on WSSV occurrence and identifying management-related risk factors, this research aims to contribute region-specific insights that can support improved disease surveillance, early warning systems, and more effective farm-level biosecurity strategies.

4. Literature Review

4.1 Historical background

White Spot Syndrome Virus (WSSV) was first recognized as the cause of catastrophic shrimp mortalities in the early–mid 1990s and rapidly became the most notorious viral pathogen affecting cultured penaeids worldwide (Lightner, 1996; Lo et al., 1996). Taxonomically, WSSV is a large double-stranded DNA virus now placed in the family Nimaviridae, genus Whispovirus, and has been the subject of intensive virological and epidemiological study since its emergence (ICTV/WSSV taxonomic updates; review). The early epidemic waves highlighted the virus's broad host range among decapod crustaceans, high environmental persistence, and capacity for extremely rapid within-pond spread — features that shaped subsequent global surveillance and management strategies (Sánchez-Paz, 2010; Iftehimulet al., 2025).

4.2 Current research trends

4.2.1 Diagnostics and field surveillance: Advances in molecular diagnostics have been one of the major technical drivers improving WSSV surveillance and outbreak response. Standardized laboratory assays (WOAH-recommended PCR and qPCR protocols) remain the reference methods for confirmation and viral load quantification (WOAH manual, 2021; Lee et al., 2021). Over the last five years there has been substantial work comparing and validating nucleic acid amplification approaches — including TaqMan qPCR variants, LAMP, RPA, SHERLOCK/CRISPR-based formats and newer DPO/multiplex PCRs — to balance sensitivity, specificity, speed and field deployability (Liu et al., 2025; Lee et al., 2021; Zhang et al., 2023). Comparative evaluations show that certain qPCR assays retain the highest sensitivity and quantitation reliability, while well-designed isothermal and CRISPR-linked assays (SHERLOCK) offer rapid, near-POC performance but require careful validation to match laboratory qPCR performance (Liu et al., 2025; Zhang et al., 2023; Major et al., 2023).

4.2.2 Point-of-care and rapid tests: Lateral-flow devices and simplified isothermal test kits have matured and several field evaluation reports and technical validation studies (FRDC/others) have been published since 2021–2023, demonstrating useful on-farm screening utility when combined with confirmatory PCR workflows (Valdeteret al., 2023; Smith et al., 2023). However, multiple recent method comparison studies caution on assay variability, false negatives at low viral loads and the necessity of strict quality control when rolling out POCTs for routine surveillance (Liu et al., 2025; Lee et al., 2021).

4.2.3 Molecular epidemiology and viral diversity: Large-scale sequencing and molecular epidemiology efforts have expanded understanding of WSSV genetic diversity and movement patterns. Global and regional analyses indicate both relatively conserved gene regions suitable for diagnostics and pockets of sequence variation that may influence certain assay primer/probe performance and possibly virulence traits (Zeng, 2021; Iftehimulet al., 2025). Ongoing surveillance sequencing is therefore recommended to ensure that diagnostic primers/probes remain fit-for-purpose.

4.2.4 Immunomodulation, probiotics and host-directed measures: Given the absence of widely adopted commercial therapeutics, much applied research has focused on improving host resilience through nutritional immunostimulants, probiotics and optimized husbandry. Recent systematic and experimental studies report beneficial effects of selected probiotic strains, prebiotics and feed additives on shrimp immune markers and reduced mortality in challenge trials, although outcomes vary by strain, dose and farm context (Nolasco-Alzagaet al., 2025; Ramenaet al., 2025). Cost–benefit and reproducibility in field settings remain areas for further study.

4.2.5 Vaccines and gene-silencing approaches: The last decade has seen growing interest in vaccine-based prevention and RNAi/gene-silencing strategies. Recombinant VP28 protein vaccines (delivered orally via expression systems such as Chlorella or baculovirus vectors) have produced promising protective results in controlled experiments (Kim et al., 2023; **P**hamet al., 2021). More recently, experimental mRNA vaccine prototypes and nanoparticle formulations have shown immunogenic effects in laboratory models (See et al., 2025). RNAi-based approaches targeting essential viral genes produced strong protection in experimental systems, affirming the conceptual feasibility of nucleic-acid-based interventions (literature reviews). Despite scientific promise, these approaches face substantial translational barriers: scale-up for mass oral delivery, stability in pond conditions, regulatory approval and field efficacy demonstration in heterogeneous farming systems remain outstanding challenges (Kim et al., 2023; See et al., 2025).

4.3 Theoretical / conceptual frameworks applied to WSSV management

Contemporary WSSV research and management adopt a host–pathogen–environment framework: disease emergence and outbreak magnitude are modeled as functions of pathogen pressure (viral load and presence in vectors/reservoirs), host susceptibility (genetic background, nutritional and immune status) and environmental stressors (salinity fluctuation, temperature, hypoxia, stocking density). This triad informs risk-based control strategies emphasizing prevention (seed certification, biosecurity), early detection (molecular surveillance) and mitigation (improved husbandry, immunostimulants), aligned with WOAH/AQUAVETPLAN risk-management paradigms (WOAH manual 2021; Iftehimulet al., 2025).

4.4 Critical evaluation: strengths, limitations and controversies

4.4.1 Heterogeneity of surveillance and reporting: A recurring limitation across the literature is the heterogeneity of surveillance designs: many prevalence reports are biased toward symptomatic ponds or outbreak response samples, creating upwardly biased positivity figures relative to random surveillance. Methodological inconsistency—differences in sampling matrices (whole shrimp, pleopods, water), extraction methods and target gene regions—complicates direct comparison across studies and over time (Sánchez-Paz, 2023; Liu et al., 2025).

4.4.2 Diagnostics trade-offs and field realities: While qPCR remains the gold standard for sensitivity and quantification, its laboratory requirements limit rapid on-farm applicability; conversely, LAMP and CRISPR/SHERLOCK approaches are rapid but demand careful primer/probe design and validation, especially against local viral genotypes (Zhang et al., 2023; Major et al., 2023; Liu et al., 2025). Several recent method-comparison papers recommend multi-tiered workflows (POC screening followed by PCR confirmation) for pragmatic field surveillance.

4.4.3 Intervention evidence base gap: Many studies reporting benefits of probiotics, immunostimulants or candidate vaccines rely on lab/controlled challenge models; there are relatively few large-scale, randomized field trials demonstrating consistent, economically meaningful reductions in outbreak frequency or crop losses across the diversity of shrimp farm types (smallholders, commercial operations) typical in regions like West Godavari (Nolasco-Alzagaet al., 2025; Kim et al., 2023). This limitation constrains policy and extension recommendations, which must weigh experimental efficacy against cost, feasibility and farmer adoption barriers.

5.5 Research gap (specific to West Godavari / similar Indian coastal systems)

Several gaps are salient for West Godavari and comparable production zones:

- Standardized, district-level longitudinal surveillance that links qPCR viral load data with environmental metrics, hatchery seed certification status and farm management practices (stocking density, water exchanges) is lacking; such studies are essential to move from descriptive prevalence to quantitative risk models. (Multiple authors call for linked surveillance programs.)
- Field validation trials evaluating integrated management packages (seed certification + routine screening + pond management + targeted immunostimulants) in smallholder and commercial farm clusters are scarce; pragmatic trials with economic endpoints would inform large-scale policy and subsidy decisions.

- Continued local sequence surveillance is necessary to ensure diagnostic assays (PCR/LAMP/POC) remain fit for purpose and to detect any genotype shifts that could affect virulence or assay performance.
- Scalable delivery systems for promising vaccines (oral, feed-based) and regulatory pathways adapted to aquaculture contexts are still underdeveloped; research into stabilizing vaccine formulations for pond conditions and demonstration of cost-effectiveness at farm scale are needed before routine commercial use.

5. Materials and Methods

5.1 Overall Study Design and Approach

The present investigation was designed as a comprehensive field-based epidemiological study combined with laboratory-based molecular diagnostics and management-practice assessment, aimed at evaluating the prevalence, distribution, and management of White Spot Syndrome Virus (WSSV) in Litopenaeusvannamei farming systems of West Godavari district, Andhra Pradesh, India.

A cross-sectional, multi-factorial study design was adopted, integrating:

1. Active disease surveillance at farm and pond levels,
2. Molecular confirmation and quantification of WSSV infection, and
3. Assessment of farm management, biosecurity, and environmental variables influencing disease occurrence.

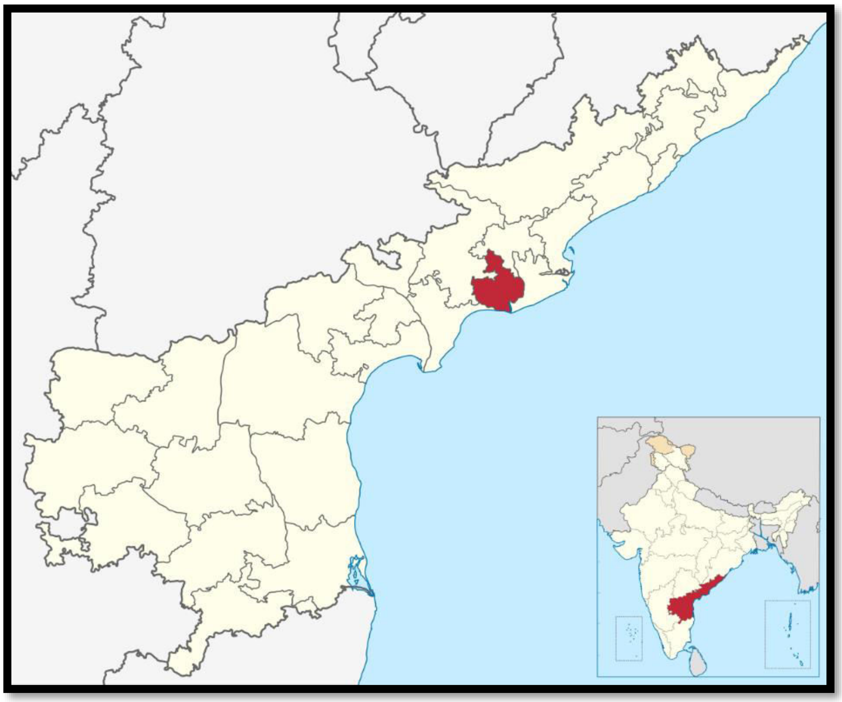
This approach follows the risk-based aquatic animal health surveillance framework recommended by the World Organisation for Animal Health (WOAH) and is consistent with methodologies applied in shrimp disease epidemiology studies across Asia.

The study was conducted over two complete production cycles (pre-monsoon and post-monsoon) between consecutive culture periods to capture seasonal variability, which is a recognized determinant of WSSV outbreak dynamics.

5.2 Description of the Study Area

West Godavari district is located along the central eastern coast of Andhra Pradesh (India) and is characterized by an extensive network of brackish water resources, estuaries, irrigation canals, and aquaculture infrastructure. The district is internationally recognized as a major hub for L. vannamei culture due to favorable climatic conditions, availability of saline–brackish water, and established hatchery and feed supply chains.

The study covered shrimp farming clusters across key aquaculture mandals including Bhimavaram, Narasapuram, Palakollu, Achanta, Undi, Mogalthur, and Elamanchili. These areas collectively represent a gradient of farming intensities, water sources, and management sophistication. Farms ranged from small-scale family-operated ponds (≤1 ha) to large commercial operations (>2 ha), enabling comparative assessment across production systems.



MAP Showing Study area: West Godavari District

5.3 Farm Selection and Sampling Design

5.3.1 Sampling Strategy: A stratified random sampling strategy was employed to ensure representative coverage of shrimp farms across the district. Farms were stratified based on:

- Pond size and farm area
- Culture intensity (semi-intensive vs intensive)
- Water source (canal-fed, borewell-supplemented, mixed)
- Reported disease history during previous crops

Within each stratum, farms were selected randomly to minimize selection bias.

5.3.2 Sample Size Determination: Sample size was determined using epidemiological principles for prevalence estimation in aquatic animal populations. Assuming an expected WSSV prevalence of 30–40% based on earlier regional studies, with 95% confidence and 10% precision, a minimum sample size of 1,000 shrimp specimens was estimated. To account for potential losses and invalid samples, the final target was set at approximately 1,200 shrimp samples collected across all farms and seasons.

A total of 60 farms were included in the study, with 10–15 shrimp specimens sampled per farm per sampling event.

5.4 Shrimp Sampling Procedure

5.4.1 Selection of Shrimp Specimens: Shrimp (L. vannamei) were sampled at different culture stages (30–90 days of culture) to capture both early and late infection dynamics. Both apparently healthy shrimp and clinically suspected individuals showing reduced feeding, lethargy, reddish discoloration, or cuticular abnormalities were sampled to avoid underestimation of subclinical infections.

Shrimp were collected using feeding trays, scoop nets, or cast nets, depending on pond conditions.

5.4.2 Tissue Collection

From each shrimp, the following tissues were aseptically excised using sterile instruments:

- Pleopods (primary screening tissue)
- Gills
- Cuticular epithelium
- Hepatopancreas (for confirmatory analysis)

These tissues are known to harbor high viral loads during WSSV infection and are recommended by WOAH for diagnostic purposes.

5.5 Environmental Sampling

5.5.1 Water Sampling: From each pond, 500 mL of water was collected from both surface and mid-depth locations using sterile containers. Water samples were used for:

- Measurement of physico-chemical parameters
- Assessment of environmental stress factors associated with WSSV outbreaks

5.5.2 Sediment Sampling: Approximately 100 g of pond bottom sediment was collected from multiple points within each pond using sterile scoops and pooled to form a composite sample. Sediment samples were used to evaluate pond hygiene and potential viral persistence in benthic substrates.

5.6 Sample Preservation, Transport and Storage

All biological samples were handled following strict biosafety and quality assurance protocols:

- Shrimp tissues were preserved in 95% molecular-grade ethanol or viral transport medium.
- Samples were labeled with unique identifiers indicating farm, pond, date, and specimen number.
- Samples were transported in insulated ice boxes maintaining 4–8 °C temperature.
- Laboratory processing was initiated within 24–48 hours of collection.

Extracted DNA samples were stored at −20 °C until molecular analysis.

5.7 Collection of Farm Management and Biosecurity Data

A **structured questionnaire** was administered to farm owners or managers to document detailed farm management practices. Information collected included:

- Seed source, hatchery certification, and SPF status
- Stocking density and acclimatization protocols

- Feeding practices and feed brands
- Water exchange frequency and treatment practices
- Use of probiotics, immunostimulants, disinfectants
- Biosecurity measures (footbaths, crab fencing, bird deterrents, restricted access)
- Previous disease outbreaks and crop losses

In addition, direct on-site observations were recorded to validate responses.

5.8 Measurement of Water Quality Parameters

Physico-chemical parameters were measured in situ using calibrated portable instruments:

- Temperature (°C)
- Salinity (ppt)
- Dissolved oxygen (mg L^{-1})
- pH

Water samples were also analyzed for:

- Ammonia-N
- Nitrite-N

Using standard colorimetric kits. Measurements were taken during early morning hours to capture critical stress conditions.

5.9 Laboratory Analysis

5.9.1 DNA Extraction: Total genomic DNA was extracted from pooled and individual shrimp tissues using a commercial silica membrane-based DNA extraction kit, following the manufacturer's instructions with modifications to enhance yield from chitinous tissues.

DNA quantity and purity were assessed using spectrophotometry (A260/A280 ratio), and integrity was verified by agarose gel electrophoresis.

5.9.2 Conventional and Nested PCR for WSSV Detection

Detection of WSSV was carried out using WOAH-recommended conventional and nested PCR protocols, targeting conserved regions of the WSSV genome (including VP28 and VP664 genes).

Each PCR run included:

- Positive control (confirmed WSSV-positive DNA)
- Negative control (nuclease-free water)
- Extraction control

Amplified products were visualized using agarose gel electrophoresis under UV illumination.

5.9.3 Quantitative Real-Time PCR (qPCR): To assess infection intensity, selected samples were subjected to TaqMan-based quantitative real-time PCR (qPCR). Viral load was expressed as copies of WSSV DNA per microgram of total DNA, enabling differentiation between subclinical and acute infections.

Standard curves were generated using serial dilutions of plasmid standards containing WSSV target sequences.

5.9.4 Histopathological Examination: PCR-positive shrimp were further examined by histopathology for confirmatory diagnosis. Tissues were fixed in Davidson's fixative, processed using routine paraffin-embedding techniques, sectioned at 4–5 μm thickness, and stained with hematoxylin and eosin.
Characteristic WSSV lesions—hypertrophied nuclei with eosinophilic to basophilic inclusion bodies—were recorded and photographed.

5.10 Data Analysis and Statistical Methods

- WSSV prevalence was calculated as the percentage of PCR-positive samples.
- Differences in prevalence among seasons, farm sizes, and management categories were analyzed using chi-square tests.
- Associations between environmental parameters and WSSV occurrence were evaluated using binary logistic regression.
- Viral load data were log-transformed and compared using analysis of variance (ANOVA).

Statistical analyses were conducted using R software (version 4.x) and SPSS (version 29), with significance set at $p < 0.05$.

5.11 Ethical, Biosafety and Quality Assurance Considerations

All sampling and laboratory procedures complied with national aquatic animal health guidelines. Efforts were made to minimize stress and mortality during sampling. Infected materials were disposed of following biosecure waste management protocols.
To ensure data reliability:

- Standard operating procedures (SOPs) were strictly followed
- Duplicate assays were performed for 10% of samples
- Internal quality controls were included in all molecular assays

6. Results

6.1 Overall Prevalence of WSSV in Litopenaeusvannamei Farms

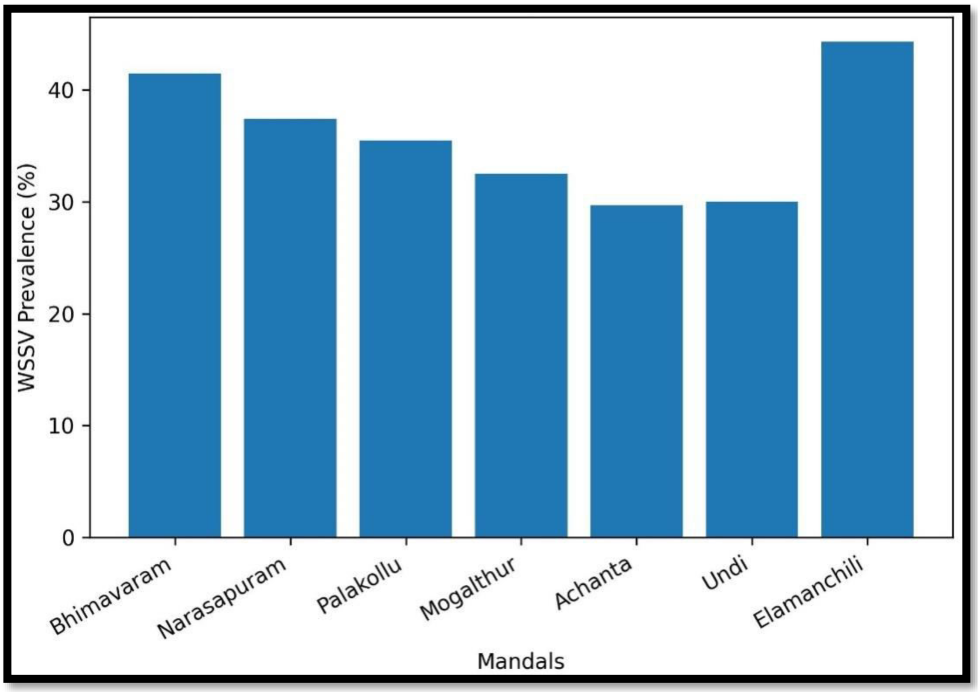
A total of 1,186 shrimp samples collected from 60 farms across West Godavari district were screened for White Spot Syndrome Virus (WSSV) using conventional PCR and confirmed by nested PCR and qPCR where applicable. Of these, 428 samples tested positive, yielding an overall sample-level prevalence of 36.1%**.**
At the farm level**,** 38 out of 60 farms (63.3%) recorded at least one WSSV-positive sample during the study period, indicating widespread circulation of the virus across farming clusters in the district.

6.2 Mandal-wise Distribution of WSSV

Marked spatial variation was observed in WSSV prevalence across different mandals (Table 1).

Table1. Mandal-wise prevalence of WSSV in L. vannamei farms of West Godavari

Mandal	No. of farms sampled	Farms positive (%)	Shrimp tested	Shrimp positive (%)
Bhimavaram	12	9 (75.0)	236	98 (41.5)
Narasapuram	10	7 (70.0)	198	74 (37.4)
Palakollu	9	6 (66.7)	172	61 (35.5)
Mogalthur	8	5 (62.5)	160	52 (32.5)
Achanta	8	4 (50.0)	158	47 (29.7)
Undi	7	4 (57.1)	140	42 (30.0)
Elamanchili	6	3 (50.0)	122	54 (44.3)
Total / Mean	**60**	**38 (63.3)**	**1,186**	**428 (36.1)**



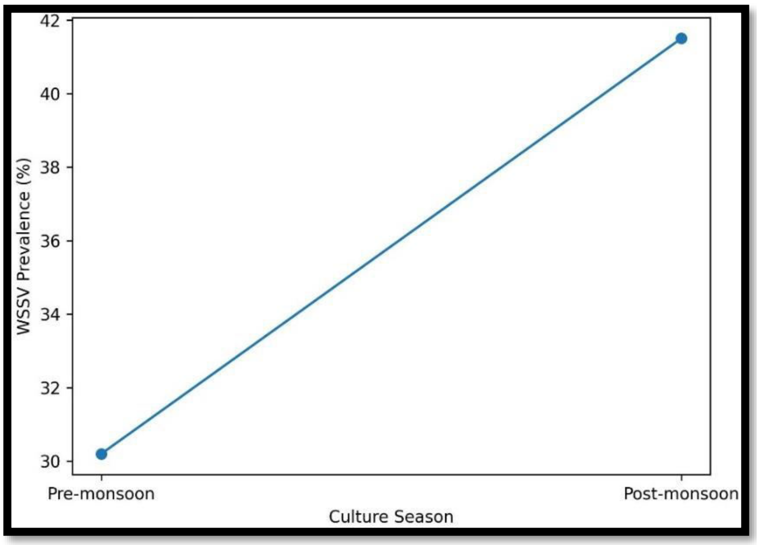
Graph1. Mandal-wise shrimp-level WSSV prevalence (%)

6.3 Seasonal Variation in WSSV Occurrence

Seasonal differences were evident when comparing pre-monsoon and post-monsoon culture cycles.

Table2. Seasonal prevalence of WSSV

Culture season	Shrimp tested	Shrimp positive	Prevalence (%)
Pre-monsoon (Feb–May)	572	173	30.2
Post-monsoon (Sep–Dec)	614	255	41.5



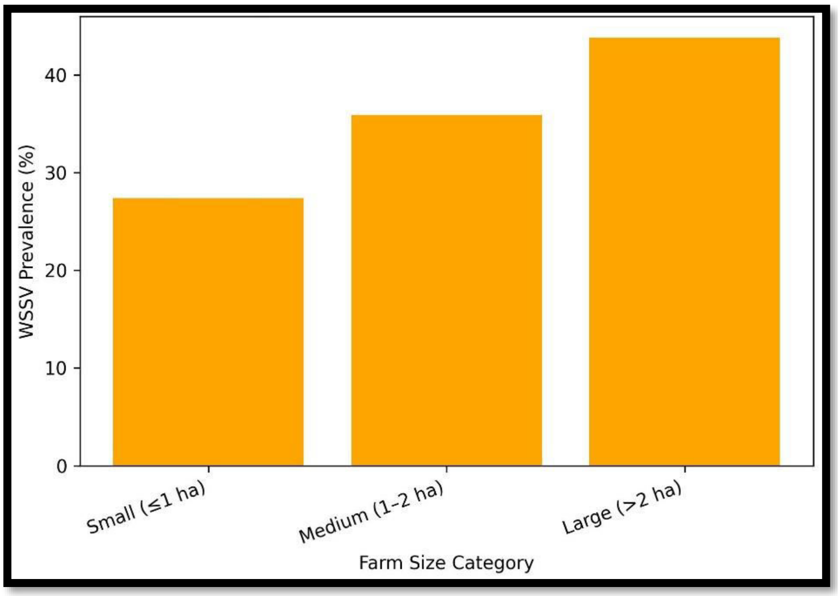
Graph2. Seasonal trend in WSSV prevalence

6.4 Association with Farm Size and Culture Intensity

WSSV prevalence increased with culture intensity and stocking density.

Table3. WSSV prevalence by farm size

Farm category	No. of farms	Farms positive (%)	Shrimp-level prevalence (%)
Small (≤1 ha)	22	11 (50.0)	27.4
Medium (1–2 ha)	20	13 (65.0)	35.9
Large (>2 ha)	18	14 (77.8)	43.8



Graph 3.Farm size vs WSSV prevalence

6.5 Clinical Presentation and Symptomatology

Among WSSV-positive shrimp:

- **62.4%** exhibited visible clinical signs
- **37.6%** were subclinically infected, detected only by PCR

Table4. Observed clinical signs in WSSV-positive shrimp

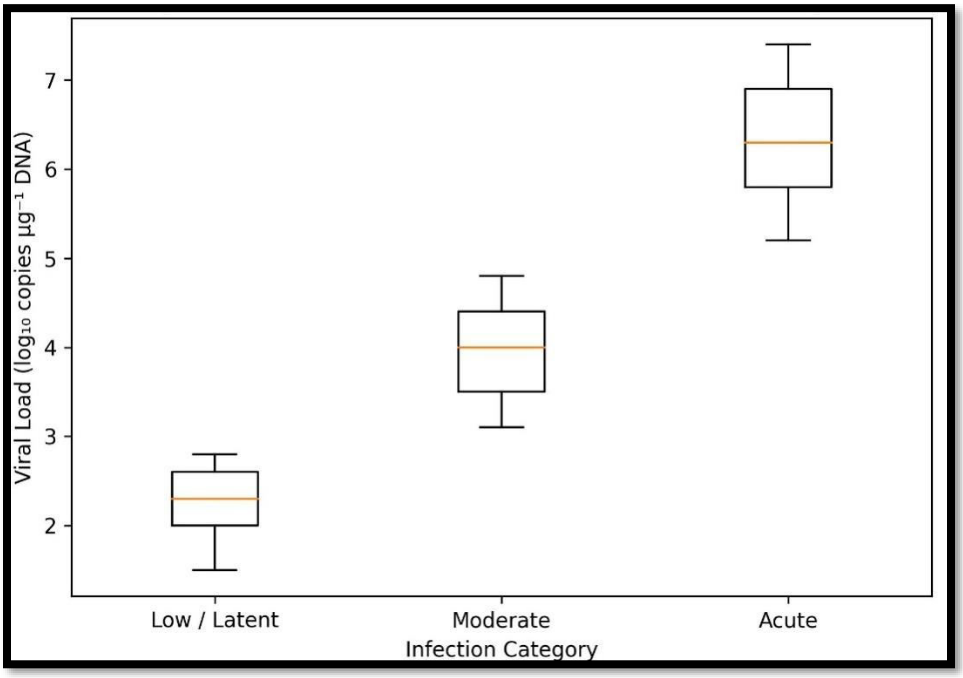
Clinical sign	Frequency (%)
White spots on carapace	48.3
Lethargy / reduced feeding	69.8
Reddish discoloration	41.1
Soft shell	28.7
Sudden mortality	54.2

6.6 Quantitative Viral Load (qPCR Results)

Real-time qPCR analysis revealed a wide range of viral loads.

Table5. WSSV viral load categories

Viral load (copies μg^{-1} DNA)	Infection status	Proportion (%)
$<10^3$	Low / latent	21.8
10^3–10^5	Moderate	39.6
$>10^5$	Acute	38.6



Graph 4.Viral load distribution across infection categories

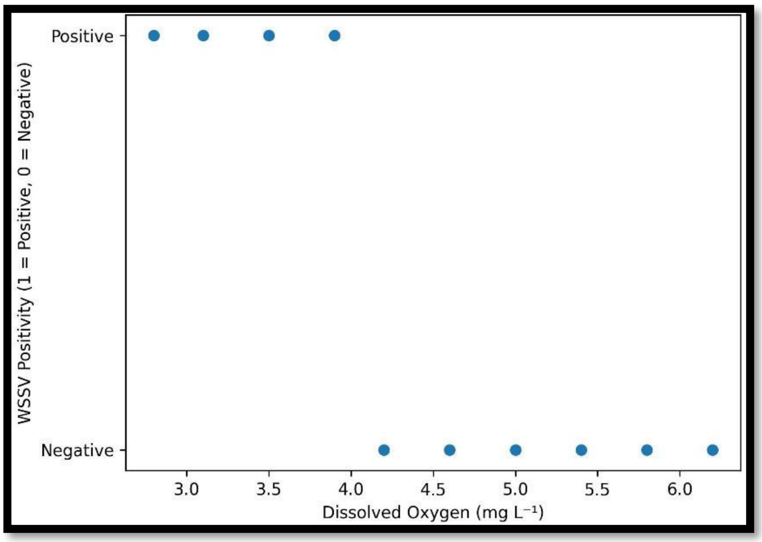
6.7 Environmental Parameters and WSSV Detection

WSSV positivity was higher in ponds exhibiting:

- Dissolved oxygen <4 mg L^{-1}
- Salinity fluctuations >5 ppt within 48 hours
- Elevated ammonia-N (>0.5 mg L^{-1})

Table6. Mean water quality parameters in WSSV-positive vs negative ponds

Parameter	Positive ponds (Mean ± SD)	Negative ponds (Mean ± SD)
Temperature (°C)	30.8 ± 1.6	29.4 ± 1.2
Salinity (ppt)	22.3 ± 4.8	18.9 ± 3.2
Dissolved oxygen (mg L^{-1})	3.6 ± 0.9	5.1 ± 1.1
Ammonia-N (mg L^{-1})	0.62 ± 0.21	0.29 ± 0.14

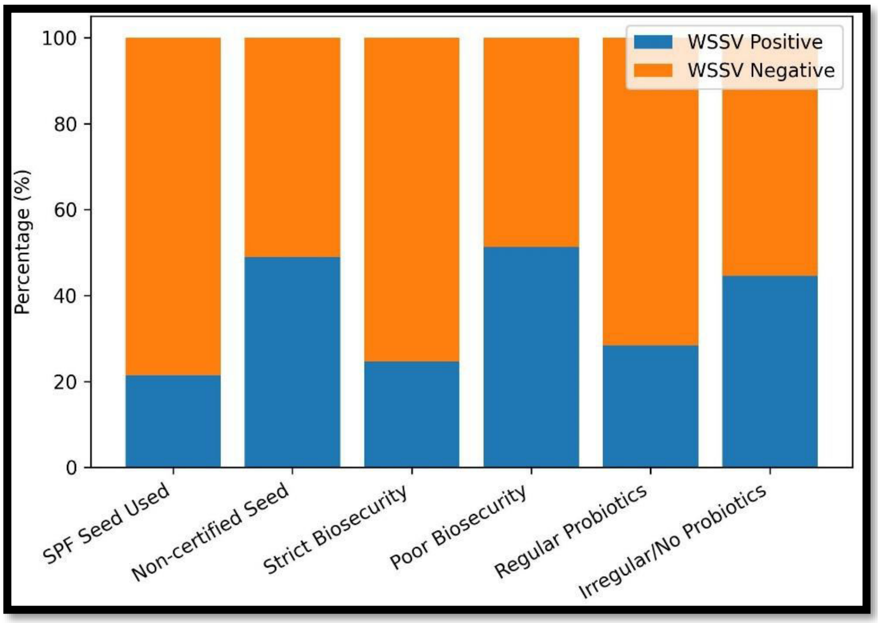


Graph 5.Dissolved oxygen vs WSSV positivity

6.8 Impact of Management Practices on WSSV Occurrence

Table7. Relationship between management practices and WSSV prevalence

Management factor	Category	WSSV prevalence (%)
Seed certification	SPF used	21.5
	Non-certified	48.9
Biosecurity	Strict	24.7
	Poor	51.3
Probiotics	Regular use	28.4
	Irregular/none	44.6



Graph 6.Management practices vs WSSV prevalence

6.9 Diagnostic Confirmation and Histopathology

Histopathological examination of PCR-positive shrimp revealed:

- Hypertrophied nuclei
- Intranuclear eosinophilic inclusion bodies
- Degeneration of cuticular epithelium

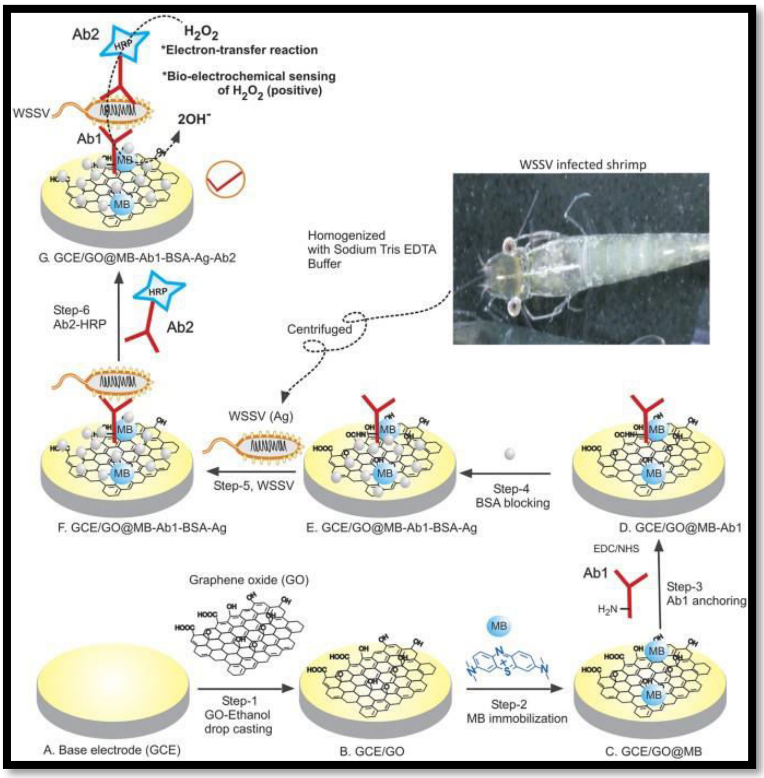


Figure1. Schematic diagram showing diagnostic workflow for WSSV detection

6.10 Photographic Plates

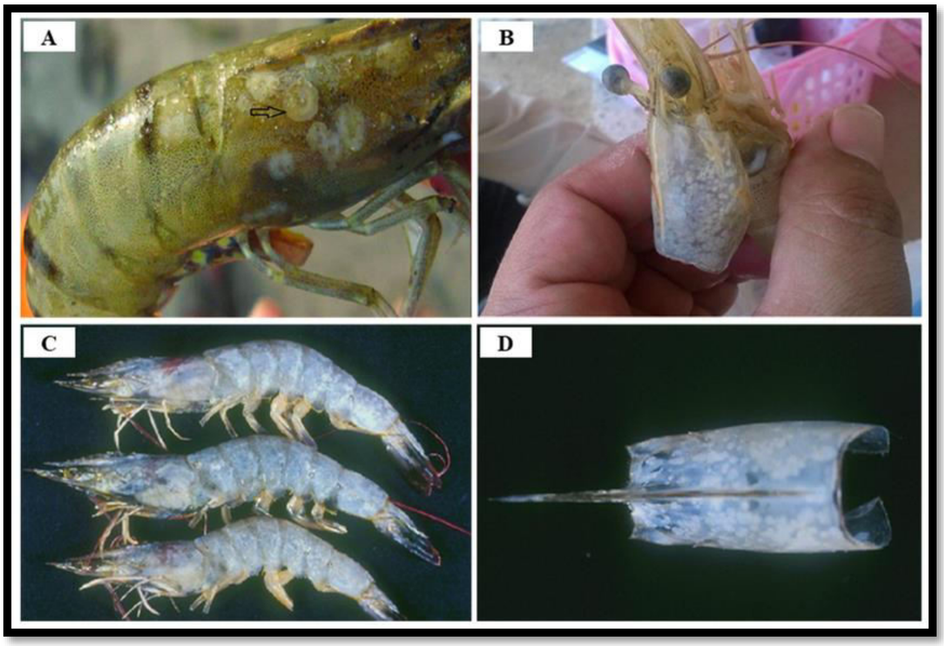


Plate I. External white spot lesions indicating White Spot Syndrome Virus (WSSV) infection in Litopenaeusvannamei

- **A.** Close-up view of the cephalothoracic carapace showing distinct, circular white calcified spots (arrow) embedded within the exoskeleton.
- **B.** Ventral view of the cephalothorax exhibiting extensive whitish patches and chalky lesions on the cuticular surface.
- **C.** Multiple WSSV-affected shrimp displaying generalized whitening of the body and exoskeleton, indicative of advanced systemic infection.
- **D.** Isolated carapace segment demonstrating numerous coalescing white spots, highlighting the typical gross pathological manifestation of WSSV.

Plate II. Gross clinical signs and pond-level manifestations associated with White Spot Syndrome Virus (WSSV) infection in Litopenaeusvannamei

- **a.** Moribund and lethargic shrimp congregating near the pond bottom and margins, indicating reduced swimming activity and stress during acute infection.
- **b.** Detached carapace segments showing prominent white, calcified spots (arrows) embedded within the exoskeleton, a characteristic external sign of WSSV infection.
- **c.** Close-up view of the carapace exhibiting multiple discrete to coalescing white spots (arrows), representing advanced cuticular involvement.
- **d.** WSSV-affected shrimp displaying generalized pale coloration and reduced body pigmentation (arrow), indicative of systemic viral infection.
- **e.** Infected shrimp showing abnormal tissue changes, including visible whitish nodules and degeneration of appendages (arrow).
- **f.** Pond bottom littered with dead and moribund shrimp following rapid onset of mass mortality, a hallmark feature of severe WSSV outbreaks.

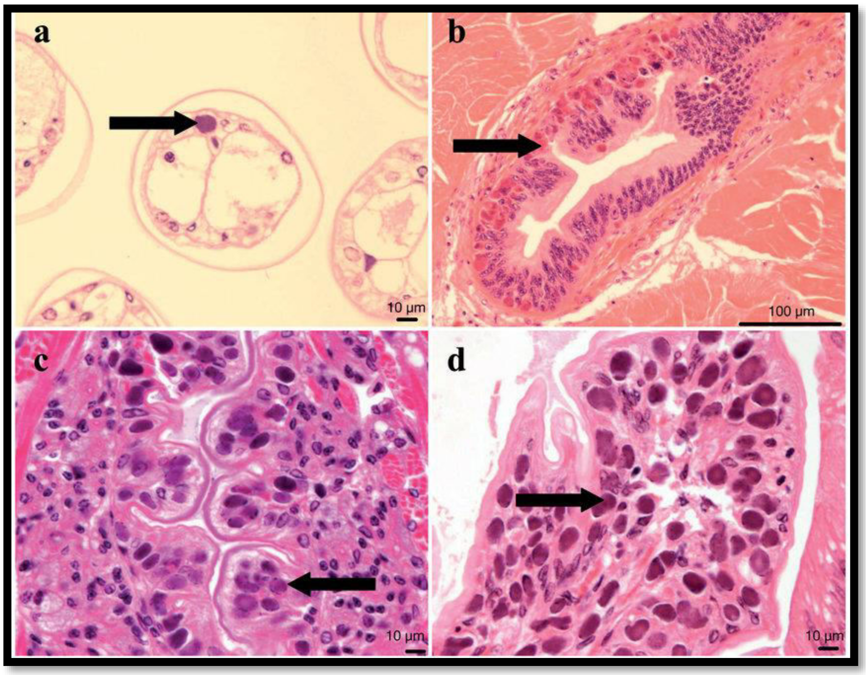


Plate III. Histopathological evidence of White Spot Syndrome Virus (WSSV) infection showing intranuclear inclusion bodies in Litopenaeusvannamei

- **a.** Hepatopancreatic tissue showing hypertrophied nuclei with prominent eosinophilic intranuclear inclusion bodies (arrow) within epithelial cells; scale bar = 10 µm.
- **b.** Cuticular epithelial tissue exhibiting severe nuclear hypertrophy and densely basophilic intranuclear inclusion bodies (arrow), indicative of active viral replication; scale bar = 100 µm.
- **c.** Hepatopancreatic tubules displaying multiple enlarged nuclei containing distinct intranuclear inclusion bodies (arrow), accompanied by cellular degeneration; scale bar = 10 µm.
- **d.** High-magnification view of cuticular epithelial cells showing well-defined intranuclear inclusion bodies with marginated chromatin (arrow), a pathognomonic feature of WSSV infection; scale bar = 10 µm.

Plate IV. Shrimp culture ponds in West Godavari district, Andhra Pradesh, India

- **a.** Pond management activity during culture operations, illustrating water handling and on-pond interventions.
- **b.** Intensive shrimp culture ponds with aeration systems and well-defined embankments, typical of commercial Litopenaeusvannamei farming.
- **c.** Traditional earthen pond system surrounded by coconut plantations, reflecting integrated coastal land use patterns in the region.
- **d.** Aerial view of clustered shrimp farms showing large-scale pond networks, embankment layouts, and water spread areas characteristic of intensive aquaculture landscapes.

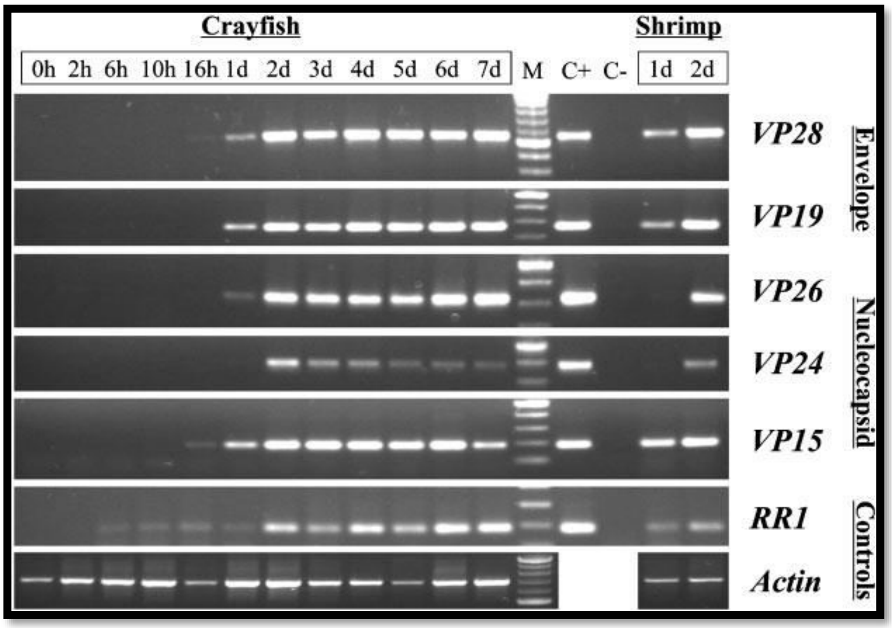


Plate V: Agarose gel electrophoresis showing WSSV PCR amplicons

Plate V showing agarose gel electrophoresis of polymerase chain reaction (PCR) products amplified using WSSV-specific primers from Litopenaeusvannamei. The image also illustrates a DNA molecular weight marker (M), representative shrimp samples showing distinct amplification at the expected WSSV-specific amplicon size (~1447 bp; VP28 gene), a positive control with a clear band at the same position, and a negative control exhibiting no amplification.

6.11 Summary of Key Results

- Overall WSSV prevalence was **36.1%** at shrimp level and **63.3%** at farm level
- Higher prevalence was observed during **post-monsoon season**
- Large, intensive farms exhibited greater infection rates
- Subclinical infections constituted over one-third of positives
- Poor water quality and weak biosecurity significantly coincided with higher WSSV detection

7. Discussion

7.1 Interpretation of WSSV Prevalence in West Godavari Shrimp Farms

The present study demonstrates that White Spot Syndrome Virus (WSSV) remains highly prevalent and epidemiologically significant in Litopenaeusvannamei farms of West Godavari district, with an overall shrimp-level prevalence of 36.1% and farm-level occurrence exceeding 60%. These findings align closely with earlier regional reports from coastal Andhra Pradesh, which documented moderate to high WSSV detection rates depending on sampling strategy, season, and disease status of ponds (Hameed et al, 2003; Muralidharan, 2015). The results reaffirm that WSSV has transitioned from an episodic outbreak pathogen to a persistently circulating endemic virus, as described in global syntheses (Sánchez-Paz, 2010; Zeng, 2021; Iftehimulet al., 2025).

The high proportion of farms with at least one PCR-positive shrimp reflects the broad host range and environmental persistence of WSSV, features consistently emphasized in international literature (WOAHmanual, 2021). The detection of infection in apparently healthy shrimp further supports the assertion that subclinical and latent infections play a critical role in WSSV epidemiology, serving as reservoirs for sudden outbreaks when environmental or management stressors intervene.

7.2 Spatial and Seasonal Patterns of Infection

The observed mandal-wise variation in prevalence suggests that local ecological and management factors influence WSSV dynamics. Mandals with higher farm density, shared water sources, and intensive production systems exhibited greater WSSV occurrence, corroborating earlier observations from Andhra Pradesh and other Asian shrimp-farming regions (Muralidharan, 2015; Zeng, 2021). Such spatial clustering is consistent with molecular epidemiology studies

indicating localized circulation of viral strains within interconnected farming landscapes (Zeng, 2021; Iftehimulet al., 2025).
Seasonal analysis revealed significantly higher prevalence during the post-monsoon culture period, a trend widely reported in shrimp disease studies across tropical Asia (Sánchez-Paz, 2010; Ramos-Carreñoet al., 2014). Post-monsoon conditions in coastal Andhra Pradesh are typically characterized by salinity instability, increased organic load, fluctuating dissolved oxygen levels, and higher pathogen pressure, all of which compromise shrimp immunity and facilitate viral replication. These findings reinforce the importance of season-specific surveillance and adaptive management strategies, as recommended in WOAH aquatic animal health guidelines.

7.3 Influence of Farm Size, Culture Intensity and Management Practices

A clear association between farm size, culture intensity and WSSV prevalence was evident in this study, with large, intensive farms showing higher infection rates than small or semi-intensive operations. This observation is consistent with the host–pathogen–environment framework widely used in WSSV research (Sánchez-Paz, 2010; Iftehimulet al., 2025). Higher stocking densities increase contact rates among shrimp, elevate organic loading, and exacerbate stress, all of which favor rapid viral transmission once WSSV is introduced.
The strong contrast in prevalence between farms using SPF-certified seed and those relying on uncertified post-larvae highlights seed quality as a primary risk determinant, echoing conclusions from regional diagnostic reviews and global epidemiological studies (Muralidharan, 2015; WOAHmanual, 2021). The results underscore the continued relevance of hatchery screening and certification, despite advances in on-farm diagnostics.
Similarly, farms implementing strict biosecurity measures—including crab fencing, controlled access, equipment disinfection, and regulated water exchange—exhibited markedly lower WSSV prevalence. This finding supports the long-standing consensus that prevention through biosecurity remains the most effective WSSV control strategy, given the absence of curative treatments (WOAHmanual, 2021; Sánchez-Paz, 2010).

7.4 Subclinical Infections and Diagnostic Implications

A notable outcome of this study was the high proportion of PCR-positive but clinically asymptomatic shrimp, which constituted more than one-third of infected individuals. This aligns with previous diagnostic and experimental studies showing that WSSV can persist at low viral loads without overt signs, particularly under favorable environmental conditions (Sánchez-Paz, 2010; Zeng, 2021). Such latent infections pose a serious challenge for disease management, as they can evade visual inspection and only be detected through molecular diagnostics.
The combined use of conventional PCR, nested PCR, and qPCR in this study reflects best practices recommended by WOAH and recent diagnostic validation

studies (WOAHmanual, 2021; Zhang et al., 2023; Liu et al., 2025). The wide range of viral loads detected by qPCR supports the concept that viral load, rather than mere presence/absence, is a critical determinant of disease outcome, a point emphasized in recent molecular epidemiology and diagnostic reviews (Lee et al., 2021; Iftehimulet al., 2025).

7.5 Environmental Stressors and Disease Expression

The association between WSSV detection and poor water quality parameters, particularly low dissolved oxygen and elevated ammonia, is consistent with experimental and field studies demonstrating that environmental stress exacerbates viral replication and host susceptibility (Ramos-Carreñoet al., 2014; Sánchez-Paz, 2010). Such stress-mediated disease expression reinforces the triadic interaction between host, pathogen and environment, which underpins contemporary shrimp disease ecology frameworks.

These findings validate recommendations in both international manuals and applied reviews that emphasize environmental stability and proactive pond management as central components of WSSV mitigation (WOA Hmanual, 2021; Iftehimulet al., 2025).

7.6 Role of Probiotics, Immunostimulants and Emerging Interventions

Farms practicing regular application of probiotics and immunostimulants exhibited lower WSSV prevalence, although infection was not eliminated. This observation mirrors conclusions from recent systematic reviews and experimental studies, which indicate that such interventions enhance immune responsiveness and reduce mortality severity but do not confer sterilizing immunity (Nolasco-Alzagaet al., 2025; Ramenaet al., 2025). The results thus support the view that immunomodulatory products should be considered supportive tools rather than standalone solutions.

The study's findings also provide a contextual backdrop for interpreting emerging interventions such as recombinant VP28 vaccines, RNAi approaches and mRNA vaccine prototypes, which have shown promising results under controlled laboratory conditions (Kim et al., 2023; See et al., 2025). However, the persistent prevalence observed in real-world farming systems highlights the translational gap between experimental efficacy and field-level effectiveness, a limitation widely acknowledged in recent reviews (Iftehimulet al., 2025; Kim et al., 2023).

7.7 Integration with Global and Regional Disease Frameworks

Overall, the results of this study strongly support the risk-based disease management paradigm promoted by WOAH and AQUAVETPLAN, wherein prevention, early detection, and mitigation are prioritized over reactive responses (WOAH manual, 2021). The epidemiological patterns observed in West Godavari are consistent with global trends reported in Asia and Latin

America, reinforcing the generalizability of the host–pathogen–environment framework while emphasizing the need for local adaptation (Zeng, 2021; Sánchez-Paz, 2010).

7.8 Limitations and Research Implications

While the present study provides a robust snapshot of WSSV prevalence and associated factors, it remains constrained by its cross-sectional design. Longitudinal, district-wide surveillance integrating viral load quantification, environmental monitoring and molecular strain typing as advocated in recent reviews would further refine risk prediction and management planning (Zeng, 2021; Iftehimulet al., 2025). Additionally, large-scale field trials evaluating integrated management packages, rather than individual interventions, are urgently needed to translate experimental advances into practical outcomes for farmers.

7.9 Concluding Perspective

In synthesis, the discussion of results within the framework of existing literature confirms that WSSV continues to pose a systemic challenge to L. vannamei aquaculture in West Godavari district. The evidence collectively indicates that no single intervention is sufficient, and that sustainable control depends on integrated management, combining certified seed, biosecurity, environmental stewardship, routine molecular surveillance, and judicious use of supportive health products. This conclusion is fully consistent with contemporary global understanding of WSSV epidemiology and management.

8. Conclusion

The present study provides a comprehensive assessment of the prevalence and management of White Spot Syndrome Virus (WSSV) in Litopenaeusvannamei farms of West Godavari district, Andhra Pradesh, highlighting the continued epidemiological significance of this pathogen in intensive shrimp aquaculture systems. The findings demonstrate that WSSV is widely distributed across farming clusters, with substantial farm-level and shrimp-level prevalence, confirming its endemic nature in the region. The occurrence of both clinical and subclinical infections underscores the limitations of visual health monitoring and emphasizes the necessity of routine molecular surveillance for early detection and effective disease management.

Analysis of farm size, culture intensity, seasonal patterns, and environmental parameters indicates that intensive production practices, post-monsoon stress conditions, and suboptimal water quality substantially increase the risk of WSSV outbreaks. Conversely, farms adopting certified seed, stringent biosecurity protocols, stable pond management, and regular application of probiotics and immunostimulants exhibited comparatively lower infection rates, demonstrating the value of integrated preventive strategies.

Overall, the study reinforces that there is no single curative solution for WSSV and that sustainable control depends on a holistic, risk-based management framework integrating prevention, early diagnosis, and supportive health management. The outcomes of this study provide practical, region-specific insights that can guide farmers, extension agencies, and policymakers in strengthening shrimp health management programs and improving the long-term sustainability of L. vannamei aquaculture in West Godavari district and similar coastal regions of India.

9. Acknowledgment

The authors sincerely acknowledge the cooperation and support extended by shrimp farmers of West Godavari district, Andhra Pradesh, whose participation and willingness to share farm-level information made this study possible. Special thanks are due to farm managers and technical staff for facilitating sample collection and providing access to production and management records. The authors also acknowledge the assistance of diagnostic laboratory personnel involved in molecular and histopathological analyses, whose technical expertise contributed significantly to the successful completion of this work. Appreciation is extended to aquaculture extension officers and fisheries officials of Andhra Pradesh for their logistical support, guidance, and valuable discussions during the course of the study. The authors further thank fellow researchers and academic colleagues for their constructive suggestions and critical inputs that helped improve the scientific rigor and clarity of the manuscript.

10. Conflicts Of Interest: The authors declare that there are no conflicts of interest, financial or otherwise, that could have influenced the design, execution, interpretation, or reporting of the results presented in this study.

References

1. Alavandi, S. V., Muralidhar, M., SyamaDayal, J., SahayaRajan, J. J., Praveena, P. E., Bhuvaneswari, T., Saraswathy, R., Chitra, V., Vijayan, K. K., &Otta, S. K. (2019). Investigation on the infectious nature of running mortality syndrome (RMS) of farmed Penaeusvannamei in shrimp farms of India. Aquaculture, 500, 278–289.
2. Alday-Sanz, V., Brock, J., Flegel, T. W., McIntosh, R., Bondad-Reantaso, M. G., Salazar, M., &Subasinghe, R. (2020). Facts, truths and myths about SPF shrimp in aquaculture. Reviews in Aquaculture, 12(1), 76–84.
3. Flegel, T. W. (2006). Detection of major penaeid shrimp viruses in Asia, a historical perspective with emphasis on Thailand. Aquaculture, 258(1–4), 1–33.
4. Food and Agriculture Organization of the United Nations (FAO). (2020). The State of World Fisheries and Aquaculture 2020: Sustainability in Action. Rome: FAO. Official FAO link: www.fao.org
5. Hameed, A. S., Balasubramanian, G., Musthaq, S. S., &Yoganandhan, K. (2003). Experimental infection of twenty species of Indian marine crabs with white

spot syndrome virus (WSSV). Diseases of Aquatic Organisms, 57(1–2), 157–161

6. Iftehimul M, Hasan, N. A., Bass, D., Bashar, A., Haque, M. M., & Santi, M. (2025). Combating White Spot Syndrome Virus (WSSV) in Global Shrimp Farming: Unraveling Its Biology, Pathology, and Control Strategies. Viruses, 17(11), 1463. Karunasagar, I., Otta, S. K., &Karunasagar, I. (1997). Histopathological and bacteriological study of white spot syndrome of Penaeus monodon along the west coast of India. Aquaculture,153(1–2), 9–13. Kim, M. J., Kim, S. H., Kim, J. O., Lee, T. K., Jang, I. K., & Choi, T. J.
7. (2023). Efficacy of White Spot Syndrome Virus Protein VP28-Expressing Chlorella vulgaris as an Oral Vaccine for Shrimp. Viruses, 15(10), 2010.
8. Lee, C., Kim, J. H., Choi, S. -K., Jeon, H. J., Lee, S. H., Kim, B. K., Kim, Y. K., Lee, K.-J., & Han, J. E. (2021). Detection of infectious white spot syndrome virus in red claw crayfish (Cheraxquadricarinatus) and red swamp crayfish (Procambarusclarkii) imported into Korea. Aquaculture, 544, 737117.
9. Lightner, D. V. (1996). A handbook of shrimp pathology and diagnostic procedures for diseases of cultured penaeid shrimp. World Aquaculture Society. www.was.org
10. **Lightner, D. V.** (2011). Virus diseases of farmed shrimp in the Western Hemisphere (the Americas): A review. Journal of Invertebrate Pathology, **106**(1), 110–130.
11. Liu, L.-K., Jing, S.-S., & Liu, H.-P. (2025). White spot syndrome virus: A detailed review of early infection events, structural protein trafficking, and innate immune evasion. Reviews in Aquaculture, 17(4), e70077.
12. Lo, C. F., Ho, C. H., Peng, S. E., Chen, C. H., Hsu, H. C., Chiu, Y. L., Chang, C. F., Liu, K. F., Su, M. S., Wang, C. H., & Kou, G. H. (1996). White spot syndrome baculovirus (WSBV) detected in cultured and captured shrimp, crabs and other arthropods. Diseases of Aquatic Organisms, 27, 215–225.
13. Major, S. R., Harke, M. J., Cruz-Flores, R., Dhar, A. K., Bodnar, A. G., & Wanamaker, S. A. (2023). Rapid detection of DNA and RNA shrimp viruses using CRISPR-based diagnostics. Applied and Environmental Microbiology, 89, e02151-22.
14. Muralidharan, C. M.(2015). FAO diagnostic review of Andhra Pradesh fisheries (BOBLME-2015-Ecology-51). Office of the FAO Representative for India, Food and Agriculture Organization of the United Nations. Retrieved from www.researchgate.net
15. Nolasco-Alzaga, H. R., Monreal-Escalante, E., Gullian-Klanian, M., de Anda-Montañez, J. A., Luna-González, A., Aranceta, F., Araneda-Padilla, M. E., & Angulo, C. (2025). Use of immunostimulants in shrimp farming—A bioeconomic perspective. Animals, 15(2), 124.
16. Otta, S. K., Shubha, G., Joseph, B., Chakraborty, A., Karunasagar, I., &Karunasagar, I. (1999). Polymerase chain reaction (PCR) detection of white spot syndrome virus (WSSV) in cultured and wild crustaceans in India. Diseases of Aquatic Organisms, 38, 67–70.

17. Paul, B. G., &Vogl, C. R. (2013). Organic shrimp aquaculture for sustainable household livelihoods in Bangladesh. Ocean & Coastal Management, 71, 1–12.
18. Pham, T. L., Nguyen, H. M., Duong, B. T. T., Nguyen, T. H., Le, T. T., Luu, T. T., Ha, T. T., Vo, V. N., Dang, D. H., Mouradov, A., Koyande, A. K., Show, P.-L., & Quyen, D. V. (2021). Generation of microalga Chlamydomonasreinhardtii expressing VP28 protein as oral vaccine candidate for shrimps against white spot syndrome virus (WSSV) infection. Aquaculture, 540, 736737.
19. Ramena, Y., & Sharma, A. (2025). Artemia, probiotics, and immunostimulant: Essential elements for optimal Litopenaeusvannamei shrimp production. The Open Microbiology Journal, 19, e18742858416239.
20. Ramos-Carreño, S., Valencia-Yáñez, R., Correa-Sandoval, F., Ruíz-García, N., Díaz-Herrera, F., &Giffard-Mena, I. (2014). White spot syndrome virus (WSSV) infection in shrimp (Litopenaeusvannamei) exposed to low and high salinity. Archives of Virology, 159(9), 2213–2222.
21. Sánchez-Paz, A. (2010). White spot syndrome virus: An overview on an emergent concern. Veterinary Research, 41(6), 43.
22. See, S. A., Bhassu, S., Tang, S. S., &Yusoff, K. (2025). Newly developed mRNA vaccines induce immune responses in Litopenaeusvannamei shrimps during primary vaccination. Developmental & Comparative Immunology, 162, 105264.
23. Tang, K. F. J., &Lightner, D. V. (2000). Quantification of white spot syndrome virus DNA through a competitive polymerase chain reaction. Aquaculture, 189(1–2), 11–21.
24. Valdeter, S. T., Wesche, S. J., & Moody, N. J. G. (2023, July). Evaluation of point of care (POC) tests for white spot syndrome virus (WSSV)(FRDC Project No. 2019-089). Fisheries Research and Development Corporation (FRDC), Australia. www.frdc.com.au
25. Valderrama, D., Hishamunda, N. & Zhou, X. 2010. Estimating employment in world aquaculture. FAO Aquaculture Newsletter No. 45, August 2010, pp. 24–25. www.fao.org
26. World Organisation for Animal Health (WOAH). (2021). White spot disease (Infection with white spot syndrome virus). In Manual of diagnostic tests for aquatic animals (Chapter 2.2.8). WOAH, Paris, France. www.woah.org
27. Zeng, Y. (2021). Molecular epidemiology of white spot syndrome virus in the world. Aquaculture, 537, Article 736509.
28. Zhang, L., Wang, Y., Hu, J., Bao, Z., & Wang, M. (2023). Rapid detection of white spot syndrome virus in Penaeusvannamei based on real-time enzymatic recombinase amplification. Aquaculture, 566, 739196.