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Analysis of the Distribution of White Spot Syndrome Virus (WSSV) in Vannamei Shrimp (*Litopenaeus vannamei*) in Bali: Water Quality and PCR Study

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Abstract. White Spot Syndrome Virus (WSSV) is a pathogen responsible for white spots, typically 0.5–2 mm in diameter, on the shrimp cephalothorax. WSSV is capable of causing mass mortality rates of up to 100% within 3 to 10 days of symptom onset. The rapid transmission and detection challenges associated with WSSV have resulted in significant economic losses for Indonesian shrimp farmers. In Bali, *Litopenaeus vannamei* (Vannamei shrimp) is the second-largest marine aquaculture commodity after seaweed, with major seed production centered in the Gerokgak and Seririt Districts of Buleleng Regency. Given the difficulty in controlling WSSV, identifying its emergence and characteristics is critical. This study aimed to determine the distribution of WSSV in Bali by examining the relationship between water quality and Polymerase Chain Reaction (PCR) detection results, and by analyzing the correlation between water quality, WSSV incidence, and mortality rates in monitoring ponds. The research focused on PCR analysis and water quality assessment of Vannamei shrimp from the Karangasem, Buleleng, and Jembrana Regencies, conducted by the Indonesian Quarantine Agency (BKI). Water quality parameters, including temperature, transparency, pH, DO, salinity, nitrite, nitrate, ammonia, and phosphate, were measured using test kits. The study detected no WSSV during the examination period. PCR analysis showed amplification products ranging from 200 to 400 bp, distinct from the standard WSSV-positive marker at 941 bp. Furthermore, all water quality samples met the criteria and compliance parameters established by the Animal, Fish, and Plant Quarantine Center (BBKKIT).

Keywords: WSSV; PCR; water quality; Vannamei shrimp; virus distribution.

I. INTRODUCTION

Vannamei shrimp (*Litopenaeus vannamei*) are widely cultivated across various regions of Indonesia, including East Java, Serang, Karawang, Tangerang, and Pasuruan [1-7]. Initially, this species was highly favored due to its perceived disease resistance. However, the cultivation sector now faces significant challenges from the White Spot Syndrome Virus (WSSV) (8–10). This pathogen is characterized by white spots, typically 0.5–2 mm in diameter, on the shrimp's cephalothorax (9). Clinical symptoms include decreased appetite, reddish-brown discoloration, lateral swimming, and lethargy near the pond surface. WSSV is highly lethal, capable of causing up to 100% mortality within 3 to 10 days of symptom onset. In Bali, where Vannamei shrimp is the second-largest marine aquaculture commodity after seaweed, this virus poses a critical threat [4].

A decline in water quality can compromise the immune system of farmed shrimp, significantly increasing their susceptibility to disease [11-15]. Suboptimal environmental conditions not only trigger disease outbreaks but may also facilitate viral mutations, altering the pathogen's morphology and characteristics [8, 9]. This

risk is particularly relevant in the Gerokgak and Seririt Districts of Buleleng Regency, North Bali, which serve as key centers for Vannamei shrimp seed production [3].

The significant activity in the shrimp trade positions the BKI quarantine facility as a strategic site for analyzing disease dynamics in Bali. Controlling the rapid spread of WSSV remains a challenge, underscoring the urgency to identify its characteristics and emergence. This study employs Polymerase Chain Reaction (PCR) because of its ability to detect trace amounts of the virus (high sensitivity) and differentiate WSSV from other viruses using specific primers (high specificity). Based on this background, this research focuses on mapping the distribution of WSSV across Bali.

II. METHODS

A. Research Time and Location

This study was conducted from December 1, 2024, to February 1, 2025. Laboratory analysis regarding WSSV infection in Vannamei shrimp was performed at the Bali Animal, Fish, and Plant Quarantine Center (BBKHIT). The facility is located at Jl. Raya Sesetan No. 312 / Jl. Raya Benoa No. 20, Pedungan, South Denpasar, Bali, 80222.

B. Research Design

This study employed a quantitative, exploratory research design. Exploratory research focuses on hypothesis development rather than hypothesis testing, serving as a foundation for formulating problems for future investigations. The study's scope included molecular detection via conventional PCR and water-quality assessment of *Litopenaeus vannamei* in the Karangasem, Buleleng, and Jembrana Regencies. Conventional PCR was utilized due to equipment availability at the BBKHIT Bali laboratory. It is acknowledged that this method may have limitations in detecting low-level infections compared to more advanced techniques (16–21). Water quality parameters, including temperature, transparency, pH, Dissolved Oxygen, salinity, nitrite, nitrate, ammonia, and phosphate, were measured using standard test kits. This study did not compare the accuracy or sensitivity of the conventional PCR method with other molecular methods, such as Real-Time PCR (qPCR) or Loop-mediated Isothermal Amplification (LAMP).

C. Sample Population

The study involved sampling from three distinct locations (Karangasem, Buleleng, and Jembrana Regencies) within the BBKHIT Bali monitoring network. These areas were selected as they represent the primary centers of shrimp production in Bali. From each location, 10 Vannamei shrimp were randomly selected. The sample size was determined using the Amos Table (Table 1), based on the estimated population per pond and the target prevalence. This calculation assumed a reference population of 50 individuals per sampling site.

TABLE 1
SAMPLE SIZE DETERMINATION BASED ON SNI 7306:2006

	Alleged Revolution						
Population	2 %	5 %	10 %	20 %	30 %	40 %	50 %
≤50	46	29	20	10	7	5	2
100	76	43	23	11	9	7	6
250	110	49	25	10	9	8	7
500	127	54	26	10	9	8	7
1000	136	55	27	10	9	9	8
2500	142	56	27	10	9	9	8
5000	145	57	27	10	9	9	8
10.000	146	57	27	10	9	9	8
100.000	147	57	27	10	9	9	8
≥100,000	150	60	30	10	9	9	8

D. Data Collection by:

1. Laboratory Observation: Direct documentation of laboratory protocols was performed during the PCR process. This included recording all stages from sample preparation, DNA extraction, and amplification, to the analysis of electrophoresis results.
2. Literature Review: Relevant studies were collected and synthesized to provide a comparative framework for the current research findings.

E. Data Analysis

Data analysis focused on three key aspects:

1. PCR Interpretation: WSSV detection was determined based on electrophoresis results, strictly adhering to the protocols established by the World Organisation for Animal Health (OIE).
2. Spatial Analysis: Utilized to map the distribution of WSSV incidence across the study areas.
3. Statistical Analysis: Regression analysis was employed to examine the correlation between WSSV incidence and water quality parameters.

III. RESULTS AND DISCUSSION

A. Sample Characteristics

Table 2 presents the characteristics of the Vannamei shrimp samples collected from monitoring sites in the Karangasem, Buleleng, and Jembrana Regencies.

TABLE 2
CHARACTERISTICS OF VANNAMEI SHRIMP SAMPLES FROM MONITORING LOCATIONS

No	Lots of samples	Types of Media Operators	Sampling Location	Body length (cm)	Body Weight (gr)
1.	10 Fish	Vannamei Shrimp (*L.vannamei*)	Village. Bugbug Karangasem District	3.5 – 4	1,5 – 2
2.	10 Fish	Vannamei Shrimp (*L.vannamei*)	Village. Tejakula District Wall	0,1 – 1	0,1 – 0,5
			Patas Village, Gerokgak District	0,5 – 1,5	0,1 – 0,5
3.	10 Fish	Vannamei Shrimp (*L.vannamei*)	Pen	5 – 10	0,5 – 6,5
			Village. Letang National District	4,6 – 10	0,5 – 5,5

B. *Water Quality Results*

The results of the water quality tests for three monitoring samples from Karangasem, Buleleng Regency, and Jembrana Regency are shown in Table 3.

TABLE 3.
WATER QUALITY TEST RESULTS

Parameters	BBKHIT Optimal Standards	Test Results		
	Shrimp/ Brackish water	Karangasem	Stuttgart	Jembrana
Temperature (°C)	27 – 31	30	30	30
Brightness (cm)	30 – 45	40	40	40
pH	7.5 – 8.5	7	7	7
DO (mg/L)	>4	4	4	4
Salinity (g/l)	5 – 40	25	25	25
Nitrite (mg/L)	0.001	0,5	0	0
Nitrate (mg/L)	< 10	25	25	50
Ammonia (mg/L)	< 0.1	0.25	0.5	0.2
Pospat (mg/L)	0.1	1	0.25	1

According to Chanratchookl (1998) in Shirley [16], A decrease in water quality can reduce the immunity of cultivated shrimp, because of such conditions, the opportunity for disease attacks on shrimp is very large. Based on the statement, it is stated that poor water quality makes the ecosystem in shrimp farming ponds trigger various diseases, an environment that is not in accordance with the parameters makes the virus undergo gene mutations by changing its shape and nature. Each water quality parameter has a relationship between the others [16].

Each water quality parameter has a relationship between the other parameters. According to Salinity can affect the nitrification and denitrification process, which can change the concentration of nitrate and nitrite, affect the balance between ammonia (NH3) and ammonium (NH4+), which can impact ammonia toxicity to shrimp, salinity also affects the availability of phosphates, which can impact shrimp growth and ecosystem balance. This is evidenced in Figure 2 Graph of the relationship of each water quality parameter [17].

C. *Laboratory Examination*

Based on the results of laboratory examinations, vannamei shrimp samples taken from Karangasem, Buleleng Regency, and Jembrana Regency found that the WSSV virus was negative (-) (Figure 1). The PCR detection results between the three study sites showed similarities because the average PCR result was at the 400 bp marker, and there were no other graphs other than positive control and samples, the sample is declared negative if it is more and less than 941 bp.

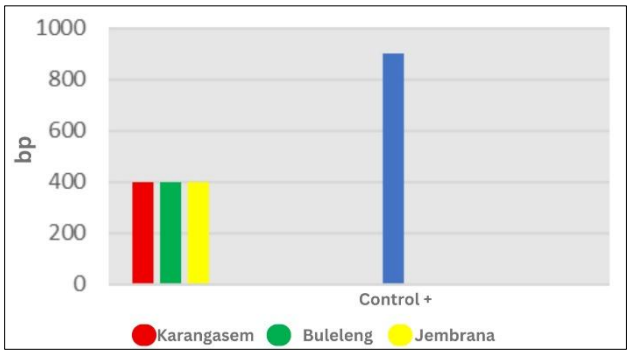

Figure 1. PCR detection results

D. *Distribution Map*

The research data indicates that, to date, the studied areas in Bali remain free from WSSV infection. While no WSSV was detected, established literature suggests a strong correlation between water quality and disease outbreaks. According to BBKHIT (2024), diseases can be triggered by high concentrations of organic matter (derived from feed, fertilizer, and molasses), as well as fluctuations in temperature, salinity, pH, and low plankton diversity.

In this study, parameters such as temperature, transparency, pH, DO, salinity, and nitrite were found to be within the optimal range compliant with BBKHIT standards. Conversely, levels of nitrate, ammonia, and

phosphate were notably high. This elevation is attributed to the accumulation of organic matter from uneaten feed, leading to sedimentation and the release of toxic compounds into the pond ecosystem. The spatial distribution map of the sampling sites in Karangasem, Buleleng, and Jembrana, verified by the BBKHIT Center Bali laboratory results, is presented in Figure 2.

Figure 2. Center of shrimp culture and sampling location

Discussion

Shrimp Health Status and WSSV Detection Based on surveillance conducted across three primary production centers (Karangasem, Buleleng, and Jembrana Regencies), this study indicates that the *Litopenaeus vannamei* populations in these regions are currently free of White Spot Syndrome Virus (WSSV) infection.

Molecular analysis utilizing Polymerase Chain Reaction (PCR) corroborated these clinical observations. Electrophoresis results showed amplification products (bands) at approximately 400 bp. This molecular weight is distinct from the standard WSSV positive control, which typically runs at 941 bp according to BBKHIT protocols. The appearance of non-specific bands at 400 bp suggests the absence of WSSV genetic material, with the observed bands likely representing host DNA or other non-target artifacts. Consequently, these findings confirm that no active outbreak occurred at the study sites during the sampling period (December 2024 – February 2025).

Water Quality Analysis: Water quality is a pivotal determinant of shrimp's defense mechanisms against pathogens. *In situ* measurements indicated that key physico-chemical parameters, specifically temperature (30°C), transparency (40 cm), pH (7), Dissolved Oxygen (4 mg/L), salinity (25 ppt), and nitrite (0–0.5 mg/L), were maintained within the optimal ranges established by BBKHIT standards. The stability of these environmental conditions is critical, as water quality degradation can compromise the shrimp's immune system, thereby increasing susceptibility to disease [16, 22-25].

However, anomalies were detected in nutrient concentrations. Nitrate, ammonia, and phosphate levels were elevated, exceeding optimal thresholds at several monitoring locations. Ammonia and Nitrate: Elevated concentrations of ammonia and nitrate are attributed to the accumulation of organic load from uneaten feed, fecal matter, and fertilizer application. Phosphate: Similarly, high phosphate levels indicate eutrophication resulting from excessive nutrient input into pond systems.

Relationship between Water Quality and Disease Absence: Despite elevated organic pollution indicators (ammonia, nitrate, and phosphate), the absence of WSSV detection suggests that environmental stressors alone are insufficient to precipitate an outbreak in the absence of the primary pathogen [26-29]. Nevertheless, these conditions serve as an early warning. As established in the literature, suboptimal environmental conditions may facilitate viral mutation or enhance virulence if the pathogen is introduced into the system [30-33].

The absence of disease in this study is likely underpinned by the maintenance of critical stress parameters (DO, pH, and Temperature) within ideal limits, alongside the implementation of biosecurity measures that effectively prevented the entry of viral vectors. However, the high organic burden poses a latent risk of triggering plankton blooms or ammonia toxicity if not mitigated through improved waste management and water exchange protocols.

Implications of Findings: These negative results confirm that the monitored regions in Bali, specifically Karangasem, Buleleng, and Jembrana, remain secure zones for *Litopenaeus vannamei* seed production and grow-out operations during the study period. However, evidence of organic accumulation underscores the urgent need for refined waste management strategies and feed efficiency optimization to prevent drastic deterioration in water quality, which could otherwise predispose the system to catastrophic disease outbreaks.

IV. CONCLUSIONS

Based on the samples analyzed during the study period, no WSSV infection was detected in *Litopenaeus vannamei* populations in Bali. Samples collected from Karangasem, Buleleng, and Jembrana Regencies showed negative PCR results, indicated by the absence of the specific 941 bp band during electrophoresis. Furthermore, water quality in the aquaculture ponds met optimal standards, which likely helped prevent the virus's growth and spread. These

findings suggest that effective surveillance and biosecurity measures successfully prevented disease entry and development during the monitoring period. However, continuous surveillance is necessary to ascertain the long-term regional health status.

Limitations: This study encountered several limitations. First, the short duration of the research restricted the scope of field sampling. Second, the limited availability of extraction materials and challenges in maintaining the cold chain (sub-zero storage) pose risks of sample degradation, potentially affecting the accuracy of electrophoresis results. Additionally, environmental conditions at the time of sampling may have been less conducive to viral spread, or viral loads may have been too low (latent infection) to be detected by conventional PCR methods.

Recommendations: To improve future investigations, several measures are recommended:

1. Evaluation of Sampling: The determination of sampling locations and sample sizes should be re-evaluated to ensure better representation.
2. Laboratory Readiness: Preparation of laboratory equipment, reagents, and analytical protocols must be optimized prior to study execution.
3. Secondary Data Integration: It is crucial to collect secondary data, including test results from local farmers during outbreaks and reports from other laboratories in Bali or the BBKHIT Center. This data will enhance the accuracy of the WSSV distribution map.
4. Advanced Diagnostics: The use of more sensitive diagnostic tools, such as Real-Time PCR (qPCR) or Loop-mediated Isothermal Amplification (LAMP), is recommended to detect low-level or latent infections.
5. Longitudinal Studies: Future research should cover longer periods (e.g., both dry and wet seasons) and analyze pond management practices such as feeding regimes, probiotic use, and disinfection to provide a comprehensive reference for WSSV control.

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