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DETECTION OF WHITE SPOT SYNDROME VIRUS (WSSV) IN POST LARVAE OF VANAME SHRIMP (*Litopenaeus vannamei*) IN THREE DIFFERENT HATCHERY

Deteksi White Spot Syndrome Virus (WSSV) Pada Post Larva Udang Vaname (*Litopenaeus vannamei*) di Tiga Hatchery Berbeda

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ABSTRACT

Vannamei shrimp (*Litopenaeus vannamei*) is a primary aquaculture commodity with an export contribution of up to 85%. However, its production faces challenges due to disease outbreaks, particularly White Spot Syndrome Virus (WSSV). This study aimed to detect the presence of WSSV in post-larvae of vannamei shrimp using the Nested-PCR method in three different hatcheries. The methodology included sample preparation, DNA extraction, DNA amplification through Nested-PCR, electrophoresis, and DNA visualization. The results showed that in the first week of testing, all samples tested negative for WSSV, as no DNA bands appeared at 941bp. However, in the second week, one sample (C5) tested positive for WSSV, indicated by the presence of a DNA band at 941bp, while the other samples remained negative. The prevalence of WSSV infection in this study reached 16.67%. The DNA concentration ranged from 94.5 ng/µL to 166.5 ng/µL, with a purity level between 1.72 and 1.86. These findings suggest that the Nested-PCR method is effective in detecting WSSV presence in vannamei shrimp, providing reliable detection rates.

Keywords: DNA, PCR, Post-larvae, Vannamei

ABSTRAK

Udang vaname (*Litopenaeus vannamei*) merupakan komoditas perikanan utama dengan kontribusi ekspor mencapai 85%. Namun, produksi udang ini mengalami tantangan akibat serangan penyakit, salah satunya White Spot Syndrome Virus (WSSV). Penelitian ini bertujuan untuk mendeteksi keberadaan WSSV pada post larva udang vaname secara molekular menggunakan metode Nested-PCR di tiga hatchery berbeda. Metode yang digunakan meliputi preparasi sampel, ekstraksi DNA, amplifikasi DNA dengan Nested-PCR, elektroforesis, dan visualisasi DNA. Hasil penelitian menunjukkan bahwa pada pengujian minggu pertama, semua sampel negatif WSSV karena tidak terdapat pita DNA pada ukuran 941bp. Namun, pada minggu kedua, satu sampel (C5) terdeteksi positif WSSV dengan kemunculan pita DNA pada

941bp, sementara sampel lainnya tetap negatif. Prevalensi infeksi WSSV dalam penelitian ini mencapai 16,67%. Konsentrasi DNA berkisar antara 94,5 ng/µL hingga 166,5 ng/µL, dengan tingkat kemurnian DNA antara 1,72-1,86. Hasil ini menunjukkan bahwa metode Nested-PCR efektif dalam mendeteksi keberadaan WSSV pada udang vaname, dengan tingkat deteksi yang dapat diandalkan.

Kata kunci: DNA, PCR, Postlarva, Vaname

INTRODUCTION

Vaname shrimp (*Litopenaeus vannamei*) is one of the leading commodities in the aquaculture sector that has high economic value and a significant contribution to fisheries exports, reaching around 85% of Indonesia's total fishery product exports (Wardani & Novitasari, 2023). According to Diatin *et al*., (2024), the nation's total vaname shrimp production is estimated to reach 1,120,000 tons by 2023, with a growth of 7.14% per year. Although despite its enormous potential in the fishing sector, vaname shrimp farming confronts several obstacles to success, chief among them being the risk of infectious illnesses that could result in a sharp drop in yield (Wiradana *et al*., 2024).

The White Spot Syndrome Virus (WSSV) is one of the primary illnesses affecting vaname shrimp. According to Hidayat et al. (2023), WSSV is a highly pathogenic virus that kills shrimp with high mortality rates that can quickly reach 100%. This virus spreads horizontally by contaminated water or dirty farming equipment and vertically through infected parents to larvae (Fauziati & Yulianti, 2022). Shrimp with WSSV infection exhibit white spots on their body and carapace, turn reddish-pale, lose their appetite, and swim more at the water's surface before dying in large numbers (Lilisuriani, 2020).

The control of WSSV's spread in the aquaculture environment depends on early detection and precise diagnosis. Nested Polymerase Chain Reaction (Nested-PCR) is one technique that has shown promise in identifying this virus infection (Widodo *et al*., 2022). Even at very low concentrations, this method's ideal sensitivity and specificity enable the identification of viral DNA, enabling the diagnosis of infection in shrimp before any clinical signs manifest (Putra *et al*., 2020).

Even though the PCR technique has been extensively employed in earlier research to identify WSSV, there are still gaps in our knowledge of the pattern of infection dissemination and prevalence across different hatcheries with varying biosecurity and culture management systems. Therefore, the aim of this research was to use the Nested-PCR method to molecularly diagnose the amplification of White Spot Syndrome Virus (WSSV) in post-larvae vaname shrimp (*L. vannamei*) at three different hatcheries. It seems likely that the study's findings will shine a light on the prevalence of WSSV in various hatcheries and offer guidance regarding how to set up more potent biosecurity protocols to stop the spread of infection in the aquaculture setting.

METHODS

This research was conducted from August to December 2024 at the Fish Health Testing Laboratory of the Brackish Water Aquaculture Fisheries Center (BPBAP) Takalar, South Sulawesi. Post larval samples of vaname shrimp (*L. vannamei*) were taken from three different hatcheries, namely in Barru District, Pangkep District, and Takalar District.

Research Procedures

Materials and Tools

Main materials used in this study were as follows: Post larval samples of vaname shrimp (*L.* vannamei) from three different hatchery locations. Qiagen, Germany primers and lysis

buffer for DNA extraction. Ethanol 95% (Merck, Germany) solvent in DNA isolation. DEPC-treated water (Invitrogen, USA) for the preservation of RNA/DNA samples purity. TAE Buffer 1X (Promega, USA) for electrophoresis of DNA. 1.5% agarose gel (Sigma-Aldrich, USA) as a medium for the separation of DNA in electrophoresis. 6X Loading dye (Thermo Fisher Scientific, USA) for DNA visualization Reference positive and negative controls implement WSSV (IQ2000™, GeneReach, Taiwan) in DNA amplification analysis. DNA marker 100 bp (Thermo Fisher Scientific, USA) for estimating the size of the DNA bands. Each sample was amplified using PCR master mix (KAPA Biosystems, USA) with Taq DNA polymerase+dNTP mix +PCR buffer.

Some of the main equipment used in this study are: Nanodrop Spectrophotometer (Thermo Scientific NanoDrop™ 2000, USA) to measure DNA concentration and purity. Thermal Cycler PCR (Bio-Rad T100, USA) for DNA amplification process. Horizontal Gel Electrophoresis (Bio-Rad Sub-Cell GT, USA) for separation of DNA fragments. Gel Documentation System (UVP GelDoc-It™, USA) for visualization of DNA electrophoresis results. Adjustable micropipettes 0.1-10 µL, 10-100 µL, and 100-1000 µL (Eppendorf, Germany) for sampling and reagents. Vortex Mixer (IKA MS3 Digital, Germany) for sample homogenization. Microcentrifuge (Eppendorf 5424R, Germany) for phase separation in DNA extraction. Hot Plate Stirrer (IKA C-MAG HS 7, Germany) for sample incubation during extraction.

Sample Handling Procedure

1. Sample Preparation

Post-larval samples of vaname shrimp (*L. vannamei*) were collected from three different hatchery locations, namely Barru, Pangkep, and Takalar districts. Samples were taken randomly with a total of six samples. Samples were packed in oxygenated plastic and stored in ice-filled styrofoam during transportation to the laboratory to prevent DNA degradation.

2. Extraction

DNA extraction was performed using the buffer lysis method, with the following steps: Post larval shrimp were crushed in lysis buffer (500 µL), then homogenized using a vortex mixer. Samples were incubated at 95°C for 10 minutes using a hot plate stirrer. After incubation, the samples were centrifuged at 12,000 rpm for 10 minutes using a microcentrifuge to separate the supernatant. 200 µL of supernatant was transferred to a new tube and 400 µL of 95% ethanol was added, then vortexed and centrifuged again at 12,000 rpm for 5 minutes. The supernatant was discarded, while the DNA precipitate was dried and dissolved with 200 µL DEPC-treated water.

3. DNA Amplification (Nested PCR)

WSSV detection was performed using the Nested-PCR method in accordance with SNI 8094.2:2016 standard. The first stage PCR reaction used 146F1/146R1 primers with the composition used, namely 14.875 µL nuclease free water, 5 µL PCR buffer, 1.5 µL $MgCl_2$, 0.5 µL DNTP mix, 0.5 µL 146F1/F2 primers, 0.5 µL 146R1/R2 primers, 0.125 µL taq DNA Polymerase, and 2 µL DNA template. First stage PCR cycle: initial denaturation 95 ^oC for 5 minutes (1 cycle), 95 ^oC for 30 seconds, 52 ^oC for 30 seconds, 72 ^oC for 1.5 minutes (30 cycles), and final extension 72 ^oC for 5 minutes (1 cycle). The second stage nested PCR used primers 146F2/146R2 with a similar temperature program, but with annealing at 55 ^oC for 30 seconds.

4. Electrophoresis

The amplification results were analyzed using 1.5% agarose gel electrophoresis. PCR samples were mixed with 5 µL loading dye. 100 bp marker DNA, positive control, and negative control were included in the gel wells. Electrophoresis was performed with a voltage of 100V for 40 minutes in 1X TAE buffer.

Variable Observed

1. Prevalence

Infection prevalence data were calculated based on the technical guidelines for monitoring quarantine fish pests and diseases number 32/KEP-BKIPM/2015 (Latritiani *et al*., 2017):

$$\text{Prevalence} = \frac{\text{Number of WSSV Infected Locations}}{\text{Total Number of Test Sample Locations}} \times 100\%$$

2. DNA Visualization

Visualization was done using Gel Documentation System with UV transilluminator under UV light for documentation.

3. Konsentrasi dan kemurnian DNA

DNA concentration and purity were measured using a Nanodrop Spectrophotometer, with optimal values of DNA purity in the range of 1.8-2.0 (A260/A280 nm).

Data Analysis

The research data were analyzed and presented descriptively with the help of image visualization. The appearance of DNA bands is an indicator of the presence of White Spot Syndrome Virus (WSSV) in vaname shrimp. Based on SNI (Indonesian National Standard) 8094.2:2016, the appearance of DNA bands at 941bp indicates positive vaname shrimp exposed to WSSV, and if no DNA bands appear, it indicates WSSV negative vaname shrimp.

RESULT

1. Prevalence

The results of the WSSV infection prevalence test in post-larval Litopenaeus vannamei from three different hatcheries revealed significant differences in infection rates among locations. Out of 24 samples, only one hatchery—Pangkep—showed WSSV infection, with a prevalence rate of 50.00%. In contrast, no infection was detected in the Barru and Takalar hatcheries, both of which exhibited a 0.00% infection rate.

Table 1. Prevalence of WSSV infection in Vaname shrimp post-larvae

Hatchery	Number of Samples	Positif Samples	Prevalence (%)
Barru (BR)	8	0	00.00[a]
Pangkep (PK)	8	4	50.00[b]
Takalar (TK)	8	0	00.00[a]
Total	24	1	16,67

Description: Mean values followed by the same letter are not significantly different according to Duncan's multiple range test with 95% confidence interval.

Statistical analysis using Duncan's multiple range test (P>0.05) indicated that the prevalence in Pangkep was significantly higher than in Barru and Takalar (denoted by different superscripts a and b). These findings suggest that the Pangkep hatchery may have environmental or management factors conducive to viral transmission, such as suboptimal biosecurity measures or fluctuating water conditions. Meanwhile, the absence of infection in Barru and Takalar indicates effective biosecurity implementation, such as the use of Specific Pathogen-Free (SPF) fry and controlled water filtration systems.

2. DNA Visualization

Detection of the presence of WSSV was done through agarose gel electrophoresis analysis after amplification using the Nested-PCR method (Fitri *et al*., 2021). Electrophoresis results showed that in the first week of testing, all samples showed no DNA band at 941 bp, indicating that no WSSV infection was detected. However, in the second week of testing, one of the samples from the Pangkep hatchery showed the appearance of a DNA band parallel to the positive control (941 bp), indicating WSSV infection with a high viral load.

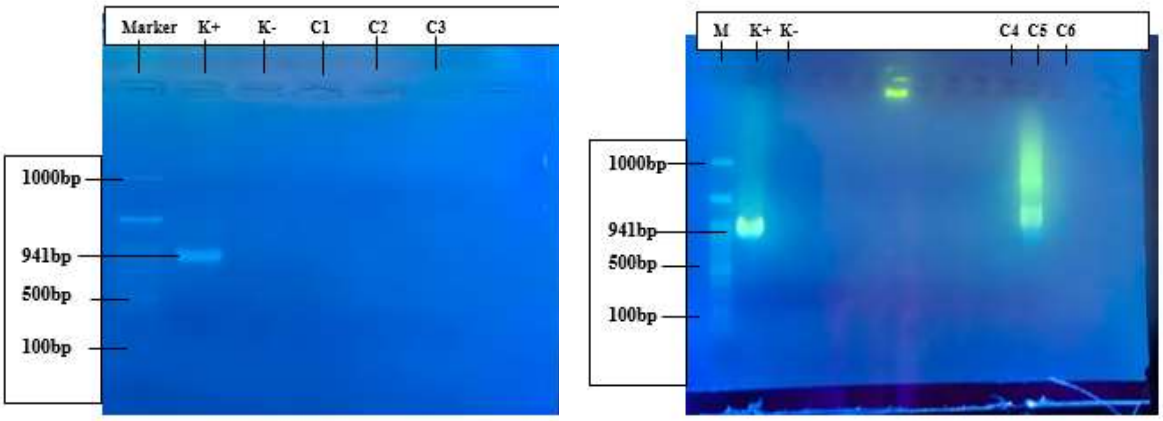

Figure 1. DNA visualization of WSSV negative research sample (A) and WSSV positive research sample (B)

3. DNA Consentration and Purify

Measurement of DNA concentration and purity using a Nanodrop Spectrophotometer showed varying results between samples. DNA purity was measured with an absorbance wavelength of 260/280 (A260/A280) (Dewanata & Mushlih, 2021). The DNA extraction results from the samples tested had a concentration range between 94.5±0,01 ng/µL to 166.5±0,01 ng/µL, with DNA purity ratio values (A260/A280) ranging from 1.72±0,01 to 1.86±0,05.

Table 2. Measurement of DNA Concentration and DNA Purity of research samples

Sample	DNA Concentration (ng/µL)	DNA Purify (A260/280nm)
BR-C1	115,4±0,02	1,80±0,04
PK-C2	94,50±0,01	1,78±0,02
TK-C3	129,1±0,00	1,72±0,01
BR-C4	148,2±0,03	1,76±0,04
PK-C5	161,2±0,01	1,86±0,05
TK-C6	166,5±0,01	1,84±0,02

DISCUSSION

The low prevalence of White Spot Syndrome Virus (WSSV) infection in the Barru and Takalar hatcheries can be attributed to the implementation of rigorous biosecurity measures, including the use of Specific Pathogen-Free (SPF) fry and comprehensive water quality management (Novriadi *et al*., 2021). SPF fry, having undergone a rigorous selection process, are certified free of specific pathogens, including WSSV. Consequently, they exhibit enhanced resistance to infections compared to uncertified fry (Khofifah *et al*., 2023). Moreover, effective water quality management, particularly the control of key physical and chemical parameters, plays a pivotal role in minimizing infection risks.

In contrast, the positive detection of WSSV in the Pangkep hatchery suggests the presence of environmental conditions conducive to viral persistence and proliferation. A key factor influencing WSSV outbreaks is water salinity and pH fluctuation. Vannamei shrimp are highly sensitive to environmental stressors, and abrupt changes in salinity and pH compromise their immune defenses, thereby increasing susceptibility to infection (Tuyen *et al*., 2024). Previous research has indicated that WSSV exhibits high transmission rates in aquaculture systems with unstable water conditions, particularly during the rainy season. Rapid decreases in salinity and temperature exacerbate infection risks, as the virus thrives under fluctuating environmental conditions (Maulana, 2023).

The effectiveness of a hatchery in preventing WSSV infection is largely dependent on the implementation of strict biosecurity protocols, encompassing water disinfection, waste management, and routine health monitoring of fry (Widodo *et al*., 2022). Hatcheries with stringent biosecurity frameworks generally report lower infection rates, as such measures mitigate potential viral transmission from external sources, including contaminated water and carrier organisms (Khofifah *et al*., 2023).

Furthermore, the findings of this study reinforce the efficacy of Nested-PCR as a diagnostic tool for WSSV detection in post-larval vannamei shrimp before clinical symptoms manifest. The principal advantages of Nested-PCR lie in its superior sensitivity and specificity, which facilitate the identification of viral DNA even at minimal concentrations (Putra *et a*l., 2020). The agarose gel electrophoresis results in this study demonstrated the presence of a DNA band at 941 bp in a single sample from the Pangkep hatchery, confirming WSSV infection with a high viral load. This observation is consistent with previous research, which has established that Nested-PCR provides a more accurate diagnosis than conventional approaches such as clinical examination and histopathology (Hidayat *et al*., 2023).

Another contributing factor to the low infection rates observed in the Barru and Takalar hatcheries is the implementation of stringent water source management. Hatcheries utilizing advanced recirculating or filtration systems typically exhibit reduced infection prevalence compared to those relying on untreated natural water sources (Novriadi *et al*., 2021). Contaminated water containing WSSV-carrying organisms, such as wild crustaceans or plankton, is a major vector for viral transmission in aquaculture settings (Fauziati & Yulianti, 2022). Thus, the adoption of effective filtration and routine water disinfection is imperative for mitigating viral contamination risks.

WSSV detection using the IQ2000™ WSSV Detection System further substantiates these findings, as a DNA band at 941 bp correlates with severe infection (>2000 copies of viral DNA), while a 333 bp band indicates mild infection (200 copies) (Abudi *et al*., 2023). In this study, the Pangkep hatchery sample exhibited a 941 bp band, confirming a high viral load. This suggests that WSSV can proliferate to substantial concentrations within shrimp tissues, leading to rapid mortality if left untreated (Hidayat *et al*., 2023).

Early detection plays a crucial role in preventing the spread of WSSV in shrimp populations. Hidayat *et al*., (2023) emphasized that agarose gel electrophoresis is a reliable

method for WSSV confirmation, especially when complemented with Nested-PCR, which offers high sensitivity for viral DNA detection. In shrimp hatcheries, early detection is vital for containment, as WSSV can rapidly disseminate through direct contact and contaminated water (Widodo *et al*., 2022). The results of this study reinforce the necessity of Nested-PCR as a primary screening method for WSSV in hatcheries.

The success of WSSV detection using Nested-PCR is contingent on the quality of DNA extracted from the sample. Mollah *et al*., (2022) indicated that optimal DNA concentration for PCR amplification exceeds 100 ng/µL. However, in this study, one sample contained 94.5 ng/µL, which, while slightly below the threshold, remained within an acceptable amplification range. Nevertheless, suboptimal DNA concentrations may increase the risk of false-negative results, particularly in the presence of protein or phenol contaminants that inhibit PCR reactions (Rosari *et a*., 2024). The majority of samples analyzed in this study exhibited DNA purity ratios (A260/A280) below 1.8, indicating potential protein contamination that could interfere with amplification. Utami *et al*., (2023) suggest that optimal DNA purity should range from 1.8 to 2.0, and additional purification steps, such as ethanol washing, can enhance DNA quality prior to amplification.

PCR amplification success is not solely determined by DNA quality but is also influenced by enzyme stability and environmental conditions. Contaminants such as proteins or phenol compounds can inhibit Taq polymerase activity, leading to suboptimal amplification or false-negative outcomes (Tanzil & Fanata, 2024). Thus, optimization of DNA extraction protocols is essential to ensure high-quality DNA suitable for diagnostic applications in WSSV detection.

In addition to DNA quality, environmental factors such as temperature and salinity fluctuations significantly impact WSSV infection dynamics. Wiradana *et al*., (2024) reported that rising water temperatures, driven by climate change, may increase the risk of WSSV outbreaks, particularly in tropical regions such as Indonesia. WSSV is most virulent at temperatures between 16°C and 26°C, whereas its activity declines above 30°C (Maulana, 2023). However, sudden temperature fluctuations induce physiological stress in shrimp, weakening their immune systems and heightening infection susceptibility. Consequently, continuous water temperature monitoring is essential, particularly during the rainy season when abrupt temperature drops can precipitate outbreaks.

Similarly, salinity fluctuations play a crucial role in WSSV transmission. Vannamei shrimp are especially vulnerable to infection following sudden decreases in salinity. Tuyen *et al*., (2024) found that reducing salinity from 30 ppt to 15 ppt increased WSSV infection risk by 40%. Salinity fluctuations disrupt shrimp osmoregulation, weakening immune defenses and facilitating infection. Thus, maintaining stable water quality parameters is paramount in minimizing WSSV transmission risks in hatcheries.

Given the substantial economic impact of WSSV outbreaks, stringent mitigation and prevention strategies must be implemented. Khofifah *et al*., (2023) reported that hatcheries enforcing strict biosecurity measures experience lower infection rates than those lacking robust environmental controls. Preventive measures include the use of SPF fry, ozone or UV-based water sterilization, and continuous water quality monitoring (Fauziati & Yulianti, 2022). Early detection through Nested-PCR and qPCR is highly recommended for tracking viral presence and quantifying viral load (Tanzil & Fanata, 2024). Finally, establishing a quarantine system for new fry prior to transfer significantly reduces WSSV introduction risks, with research indicating a 40% reduction in infection rates (Lilisuriani, 2020).

CONCLUSION

This study successfully detected White Spot Syndrome Virus (WSSV) in post-larval Litopenaeus vannamei from three hatcheries using Nested-PCR, with an infection prevalence

of 16.67%. WSSV was detected only in the Pangkep hatchery, likely due to environmental fluctuations and inadequate biosecurity. Electrophoretic analysis confirmed a high viral load, reinforcing the effectiveness of Nested-PCR for early detection. The findings highlight the importance of strict biosecurity measures and water quality management in preventing WSSV outbreaks. The absence of infection in the Barru and Takalar hatcheries demonstrates the role of Specific Pathogen-Free (SPF) fry and advanced filtration systems in disease prevention. This study also validates Nested-PCR as a reliable diagnostic tool for shrimp aquaculture. Future research should explore environmental factors influencing WSSV prevalence, apply quantitative PCR (qPCR) for viral load quantification, and develop probiotic or DNA vaccine-based biosecurity strategies to enhance disease prevention and ensure sustainable shrimp farming.

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