

Karakteristik Genetik Udang Putih (*Litopenaeus Vannamei*) di Banyuwangi dan Situbondo: Infeksi White Spot Syndrome Virus (WSSV)

Genetic Characteristics of White Shrimp (*Litopenaeus Vannamei*) in Banyuwangi and Situbondo: The Infected White Spot Syndrome Virus (WSSV)

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ABSTRACT

Water pollution caused by human anthropogenic activities has deteriorating effect on water quality for aquaculture. Therefore, the objectives of this research were to determine the water quality, population and shrimp genetic characteristic in polluted areas especially in East Java which have high virulency for WSSV outbreaks. Data exploration using surveillance technique was collecting physical and chemical water quality parameters. Shrimp samples were analyzed for morphological and genetical characteristics using specific WSSV primer ICP 11. Water quality at all sampling locations were categorized as polluted refers to ammonia and TOM concentrations. Low water quality acted as a trigger for WSSV outbreaks. Shrimp pond from Situbondo exhibited as disease carrier indicated by the presence of WSS infection based on genetic characteristics, while there are no expression on morphological characteristics.

Key words: *Vannamei shrimp, Polymerase chain reaction, Shrimp pond*

ABSTRAK

Polusi air yang disebabkan oleh aktivitas antropogenik manusia memiliki efek memburuk pada kualitas air untuk budidaya. Oleh karena itu, tujuan penelitian ini adalah untuk mengetahui karakteristik kualitas air, populasi dan genetik udang di daerah-daerah yang tercemar khususnya di Jawa Timur yang memiliki virulensi tinggi untuk wabah WSSV. Eksplorasi data menggunakan metode survei dengan mengumpulkan parameter kualitas air secara fisika dan kimia. Sampel udang dianalisis untuk karakterisasi morfologi dan data genetika menggunakan dieksplorasi menggunakan primer spesifik untuk WSSV ICP 11. Kualitas air di semua lokasi pengambilan sampel dikategorikan sebagai tercemar mengacu pada konsentrasi amonia dan TOM. Kualitas air yang rendah bertindak sebagai pemicu wabah WSSV. Tambak udang dari Situbondo ditemukan sebagai pembawa penyakit yang ditunjukkan oleh adanya infeksi WSSV berdasarkan karakteristik genetik, sementara tidak ada ekspresi pada karakteristik morfologi.

Kata kunci: Udang vannamei, PCR, Tambak udang

INTRODUCTION

Polluted environment causes shrimp disease outbreaks. According to Yanto (2006), the level of pathogenicity (virulence) of organism is different, depends on the condition of the aquatic environment in terms of water quality and the pathogenic impact. Poor water quality may cause genetic changes in vannameishrimp. Sukenda *et al.*, (2009) stated that the presence of viral diseases especially white spot disease in ponds can be tracked as DNA / RNA virus that can be duplicated by small amounts of PCR than its presence can be readily traced.

Techniques to track the presence of viral DNA can be done by several methods such as PCR. Polymerase Chain Reaction (PCR) is a technique of synthesis and DNA amplification in vitro. PCR can be used to amplify DNA segments of the million times in just a few hours. With the discovery of PCR techniques in addition to other techniques such as DNA sequencing, have revolutionized the field of science and technology especially in diagnosis of genetic diseases, forensic medicine and molecular biology (Handoyo and Rudiretna, 2000). DNA could also be used as a biomarker for early warning of disease outbreaks.

The objectives of this study were to observe the water quality condition in shrimp ponds in Situbondo and Banyuwangi, and the WSSV susceptibility using morphology and genetic characteristic using specific primer *ICP11*, as well as to know the correlation between WSSV and water quality condition. It assumed that different water quality characteristic could bring different shrimp condition and adaptation, since water resources has different physical and chemical composition. It is limited information on shrimp pond condition in East Java eventhough this areas has high contribution on shrimp export in Indonesia. In the present study, the changing pattern of

vannamei shrimp populations that live in the waters affected by pollution charges along with pollutants and toxic to the living things in the environment will be studied.

MATERIAL AND METHODS

DNA expression for vannamei shrimp was traced associated with the prevalence of WSSV, shrimp farms in one of Banyuwangi, East Java. Water quality analyzed were temperature, salinity, pH, dissolved oxygen, total organic matter, nitrite, ammonia, alkalinity and *Vibrio* presence. Water quality measurements were conducted in 2-3 different places every week for the next 4 following week 10:00 to 11:00 pm at the inlet and outlet. Method used was observational descriptive method using random sampling techniques. Vannamei shrimp sampling in Banyuwangi conducted at 2 stations were intensive and semi-intensive pond amounted to 20 shrimp tails. Shrimp taken as samples were about 1-2 months old (no age boundries). While the sample of Situbondo taken from ponds with patterns of cultivation with and without biosecurity system.

Morphological analysis using a scoring shrimp

Morphological analysis of the level of infection of WSSV in shrimp was conducted by using a code of signs or symptoms encountered. The coding in this study was based on the level of infection based on morphological characteristics of vannamei shrimp, for mild infections were scored 1 (+), infections were given a score of 2 (+ +), and severe infections were given a score of 3 (+ + +).

Analysis of Polymerase Chain Reaction (PCR)

Samples were taken of to identified WSSV infected due to morphologic analysis, then performed for Polymerase Chain Reaction (PCR) test animals to determine the positive or negative WSSV infection.

RESULTS AND DISCUSSIONS

Water quality

Water quality parameters in ponds in Situbondo and Banyuwangi were within the normal range except for high level in TOM and ammonia.

High ammonia levels indicated contamination on pond waters, range from danger to lethal level based on the National Standardization Agency (2006) less than 0.001 ppm.

According to Boyd (1990), ammonia content of 0.05 to 0.2 mg/l have hindered the growth of shrimp and other aquatic organisms. Higher concentration up to 0.45 mg/l can inhibit the rate of growth of 50 %, while the concentration of ammonia up to 1.29 mg/l was able to kill the shrimp.

Judging from the value of the total organic matter content of the pond ranged from 75.91 to 96.58 ppm, then the content of TOM either in ponds with or without biosecurity system classified as polluted by organic matter . According Cardio and Suwignyo (1980), when a body of water containing organic matter is less than 50 mg/l the influence of organic matter pollution is not significant. Adiwijaya *et al.* (2003), states that the optimal range for organic material on vannamei shrimp culture is <55 mg/l. It is also suggested by the National Standardization Agency (2006), that water quality requirements for the maintenance of organic matter on vannamei shrimp were maximum 55 mg/l.

Shrimp Morphology

Based on morphological observations on farm condition of vannamei shrimp Situbondo, that implemented biosecurity and biosecurity

classified as category 1 (no symptoms of WSSV). According to Kilawati (2011) that characteristics for the healthy vannamei shrimp were the bright body colors, no white spots on the body and quickly response after disturbance test.

In general, the types of crustaceans that exist in shrimp pond environment is potentially as susceptible and carrier of white spot disease. Naturally shrimp white spot disease, suffered through predation or cannibalism among others (Kokarkin, 1999). Clinical symptoms that arise in WSSV infected shrimp are reduced feed intake, weak, loose cuticle, pale hepatopancreas and anorexia. There are also noticeable lethargy, swim with unstable conditions, red coloration on abdomen and white spots on the carapace (Wahjuningrum *et al.*, 2006).

Genetic characterisation

In this study, the obtained DNA concentration was 52.58 - 242.95 ng / mL. DNA purity is seen at 260/280 wavelengths. According to Sambrook *et al.*, (1989) in Mulyani *et al.* (2011), the result of DNA isolation if the value is said to be pure and not contaminated with protein or RNA if the ratio of A260 / 280 is 1.8 - 2.0. The results of isolation of DNA in addition to producing isolates also contain a clear DNA smear (Figure 1).

Primer used is ICP11 (Kilawati and Darmanto 2009). The DNA amplification results were obtained to confirm the morphological conditions. The results showed that there were no symptoms of infected WSSV, but the PCR analysis showed amplification at 207 bp (ICP11 gene). this phenomenon shows a shrimp virus infection by WSSV. Viruses in infecting organisms have 2 reproductive cycles of lytic and lisogenic cycles so that shrimp can be used as carrier trait. Opinions like that Word *et al.* (2009), which states that the lytic cycle and the lysogenic cycle.

The lytic cycle is the peak reproductive cycle of the virus characterized by the death of the host cell. When the membrane cell membrane ruptures or lysis, a new virus that forms in the host cell will come out and ready to infect a new host cell. In the lysogenic cycle, the genetic material of the virus is produced in the cell without damaging its host organism.

Fegan and Clifford (2001) in Rekasana et al. (2013), states the spread of the disease, among others, is due to shrimp carrier, dead shrimp, contact with contaminated objects, water or air contaminated with pathogen carrier culture system including infected parent (seed, trunk, vector and intermediary host), host of biological carriers (as well as other intermediaries (water, automobiles, shoes, buckets, nets.) According to Sutrisno et al. (2010), in the cultivation system, the carrier includes infected host carrier pathogens (host, host, vector and host intermediaries), other biological host carriers (water, cars, buckets, shoes, nets, clothing).

Samples from Banyuwangi have differences in DNA amplification expression. Moderately infected shrimp produce amplification with a lower thickness than the heavily infected shrimp. This shows the insertion of WSSV virus in low numbers according to morphological analysis. In contrast to the heavily infected shrimp amplification products showing higher thickness, this indicates the insertion of high amount of WSSV viruses and according to what happens in the field that the sample is heavily infected with the WSSV virus.

In semi-intensive ponds found very thin DNA bands while in the intensive pool found thick ribbons. This indicates that WSSV virus accumulation in semi-intensive ponds is smaller than intensive shrimp ponds. According to Kilawati and Darmanto (2009), the thickness or quantity of different bands, ie vulnerable prawns

ICP11 is stated greater than in WSSV-resistant shrimp.

CONCLUSIONS

Water quality at all sampling locations included in the category of polluted, as indicated by the content of ammonia and high TOM at all locations. Poor water quality triggered the disease one of the detected WSSV from morphology and DNA analysis. DNA analysis results on vannamei shrimp (*Litopenaeus vannamei*) In ponds of Situbondo exhibited carrier properties as indicated by the presence of WSSV infection in shrimp vannamei DNA although the morphology did not show symptoms of infection.

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Figure

M 1 2 3 4 M 5 6 7 8

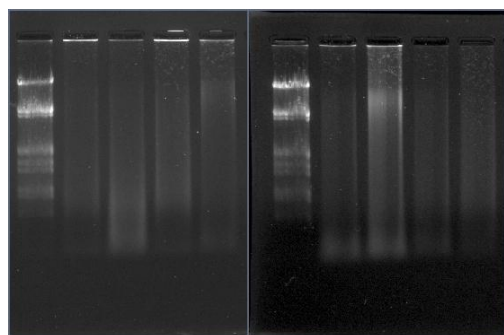


Figure 1. Electrophoresis of DNA isolated

M = marker total DNA
 1-4 = total DNA shrimp from Situbondo
 5-8 = total DNA shrimp from Banyuwangi

M C 1 2 3 4 M C 5 6 7 8

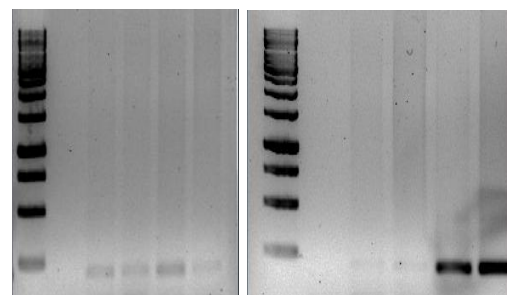


Figure 2. DNA amplification using primer ICP11

M = Marker
 C = control contain ddH₂O
 1-4 = amplifikasi DNA carrier shrimp from Situbondo
 5,6 = amplifikasi DNA mild infection from Banyuwangi
 7,8 = amplifikasi DNA high infected from Banyuwangi

