White Spot Syndrome Virus (WSSV) Detection at Traditional Ponds of *Lithopenaeus vannamei* in Pasuruan District

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Abstract

Vaname shrimp (*Litopenaeus vannamei*) is a type of shrimp that widely cultured in Indonesia. Pasuruan is one of the districts where there are many ponds that culture Vaname shrimp (*L. vannamei*) traditionally. The occurrence of a decrease in production due to White Spot Syndrome Virus (WSSV) virus attacks that cause a lot of losses for farmers, thus it needs a preventive effort by doing early warning and monitoring on the existence of the virus. The study was conducted from April to May 2018 with the aim of obtaining data on the presence of WSSV virus and its prevalence at traditional Vaname shrimp (*L. vannamei*) farms in Pasuruan District. The sampling location is located in Pasuruan District consisting of three locations namely Bangil, Kraton, and Rejoso with each has 10 ponds (total of 30 ponds sites). Detection of WSSV was using Nested PCR with shrimp bodyparts taken are swimming foot, road leg, and tail. PCR results in 848 bp and 333 bp indicated the presence of WSSV infection in the Bangil and Rejoso ponds where the prevalence rate of WSSV attack in each region differs, i.e. Bangil 0 - 15%, Kraton 0% and Rejoso 0 - 15%.

Keywords: PCR, Prevalence, Shrimp, *Vannamei*, WSSV.

INTRODUCTION

Vaname shrimp (*Litopenaeus vannamei*) is one of the many shrimp species cultivated in Indonesia. This type of shrimp dominates farming in Indonesia both intensively and traditionally. Pasuruan is one of the districts that cultivate many traditional Vaname shrimp (*L. vannamei*). The cultivation of Vaname shrimp (*L. vannamei*) developed in Pasuruan District is located in Sub-districts of Bangil, Kraton, and Rejoso [1].

But in these sub-districts, there was a decrease in Vaname shrimp production caused by White Spot Syndrome Virus (WSSV) attack on Bangil, Kraton and Rejoso. The virus is very malignant and very difficult to stop with a mortality rate of 100% in the shrimp age of cultivation between 40-60 days in just 3 to 10 days from the clinical symptoms appear. It was causing a lot of losses for the farmer so WSSV became the most serious pathogen for shrimp farming and has destroyed the shrimp industry in various countries [2-4].

Based on the above explanation, it is necessary to do a preventive effort, i.e. by doing early warning and monitoring of the existence of the virus in the environment of traditional ponds during the cultivation period. This study aims to obtain data on the presence of WSSV virus and its prevalence in traditional Vaname shrimp (*L. vannamei*) farm in Pasuruan District.

MATERIALS AND METHODS

Sampling Sites

The method used in this research is survey method with an epidemiological and observational approach. This research was conducted from April to May 2018 at traditional Vaname shrimp (*L. vannamei*) farm in Pasuruan District consisting of three locations of Sub-districts, i.e. Bangil, Kraton and Rejoso.

The number of traditional ponds sampled in Bangil sub-district are 10 ponds, Kraton Sub-District 10 ponds, and Rejoso Sub-District 10 ponds. Thus, the total samples taken are 30 ponds sites.

Shrimp sampling

Shrimp's size taken is four to seven cm long, weighing between six to 10 grams, sample age was one to two months. The number of shrimp taken from each pond is two. The sample is then preserved using 75% alcohol, put into Styrofoam/ cool box then take it to the laboratory.

WSSV tested using Nested Polymerase Chain Reaction (PCR)

Detection of WSSV was using Nested PCR with the shrimp bodyparts taken is the foot pool, foot path, and part of the tail. PCR analysis was performed in the following way:

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Extracting DNA

The 10-20 mg Vaname shrimp tissue was cut of tissue into small pieces or can also be crushed, after which it is placed on a 1.5 mL microcentrifuge tube with 180 µL ATL Buffer, plus 20 µL of K. Protein material was mixed by Vortex and incubated at a temperature of 56°C in 2-3 hours or until all of the tissue was lysis perfectly. The sample at the time of incubation can be mixed occasionally by vortex or can also be placed on thermomixer, rocking plate or water bath shaker. After vortex-mixed for 15 seconds, it added with 200 µL absolute ethanol (96-100%), vortex-mixed again until it mixed properly. The mixture in the third way is taken by using the pipette including with the sediment into the Rneasy Mini Spin column.

The mixture/sediment/pellet was placed into 2 mL collection tube. After that, the mixture/ sediment/pellet in the tube was centrifuged with a speed of 8000 rpm for approximately one minute. Then collect the tube and the solution in the tube can be removed. Then Rneasy Mini Spin collum is placed on collection tube of two mL size, after which added 500 µL buffer AW2, centrifuged for three minutes with speed 14000 rpm to dry Rneasy membrane. Collection tubes and solutions are discarded. Rneasy Mini spin column placed on a microcentrifuge tube (1.5-2 mL) added 200 µL AE Buffer for one minute and later centrifuged for one minute at 8000 rpm.

Amplification

This step was using ICP 11 Primer. The primer of mix, positive control, and negative control was placed on the cold box. Then centrifuge is done so that all the liquid collected at the bottom of the tube, which can also reduce aerosol. Each tube is placed in the thermal cycler preheat lid and set the temperature to reach 105°C. PCR is programmed to detect WSSV in order to produce a DNA Band of 848 bp at step 1 and 333 bp at step 2.

Electrophoresis

We mixed 4 µL (pipette size) in each test sample with 1 µL of diluted SYBR® Green as well as 4 µL marker plus one µl SYBR® Green of 5 µL and injected at each well at agarose carefully and slowly. Electrophoresis was conducted on 100 V for 40-60 minutes. The tool for taking electrophoresis images is a BioDoc System Imaging tool.

RESULTS AND DISCUSSION

WSSV Detection with Nested PCR

The result of the test using nested PCR found that there is WSSV in both sub-districts, Bangil and Rejoso. DNA WSSV shown with white band (as in positive control) at step 1 or band 848 and at step 2 or band 333 (Fig. 1).

Sampling and observation results with PCRs found no infection in step 1 where the positive controls were 848 bp. However, in step 2 with 333 bp positive control found some samples containing or positively has WSSV.

A healthy shrimp sample has a characteristic such as active swimming and good eating. If analysed with one step PCR, it will produce negative results. However, after testing using a nested PCR, the sample of healthy shrimp can be suspected with positive results of WSSV [5]. Detection of WSSV DNA on suspected shrimps and WSSV shrimps carriers (pests and diseases) will be more sensitive if using PCR [6].

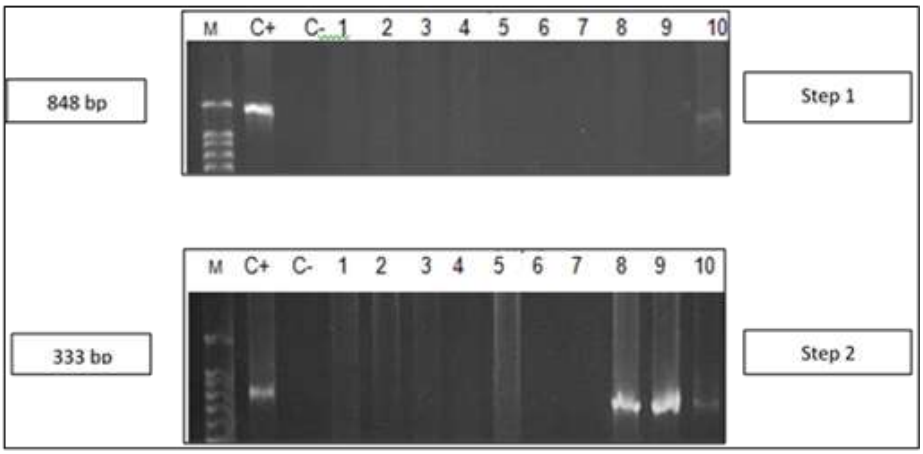

Figure 1. Results of Electrophoresis + Control at 848 bp for step 1 and 333 bp at step 2. DNA samples from shrimp in traditional farm. **Description:** Line M = Marker, C+ = Control Positive, C- = Control negative, 1 = Sample 1, 2 = Sample 2, 3 = Sample 3, 4 = Sample 4, 5 = Sample 5, 6 = Sample 6, 7 = Sample 7, 8 = Sample 8, 9 = Sample 9, 10 = Sample 10.

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White patches are a clinical symptom that the Vaname shrimp samples were attacked by the WSSV. This occurs because it is a specific lesion of WSSV [7]. This white patches assumed to be caused by the deviation on calcium metabolism accumulated in the cuticle layer on shrimp [8]. Initial diagnosis WSSV is characterized by polymorphic white spots meaning development of WSSV attacks [9]. WSSV that attacks the organs at the ectodermal and mesodermal tissues, e.g. in lymphoid, intestine, gill, skin. Whereas invaded part of the endodermal tissue is hepatopancreas [7]; in an attack on cells, the WSSV virion will use a sheath on the protein. The shell will blend with the endosome and nucleocapsid transported through the nucleus, thus attacking the membrane of the nucleus and then releasing the WSSV genome in the nucleus [10]. The WSSV gene will replicate in the cytoplasm followed by the mitochondrial damage.

The length of the WSSV DNA bands in some samples in PCR step 2 was suspected come from carrier shrimp. Through this step also found a long band of WSSV DNA bands (range of 200 bp) under positive control that we used; where the shrimp immune system contains hemocytes on shrimp hemolymph [11]. Infected WSSV has different body resistance, in which ICP 11 is a dominant gene to encode WSSV and begin expression in weaker shrimp, otherwise, shrimp with immunity to protect against disease attacks is stronger in resisting the expression of ICP 11 [12]. Factors that affect, among others, antiviral activity derived from hemocyanin which is a natural immune response to WSSV where trying to delay viral infection and also will inhibit the replication of the virus [13].

Prevalence of White Spot Syndrome Virus

Bangil Sub-district in 2018 with the number of samples as many as 20 infected shrimp samples, there are 2 positive samples of WSSV with 15% prevalence value. Whereas in Kraton Sub-district with 20 samples, the infected sample findings are 0 positive samples, thus 0% WSSV prevalence value and Rejoso Sub-district with 20 samples have the findings of infected sample 2 positive samples of WSSV, and 15% prevalence value.

Prevalence of WSSV in Bangil and Rejoso means that potential occurence of the disease was 15% while in Kraton was 0% (Table 1). The percentage of these occurrence may increased or decreased depending on factors such as water quality and environmental conditions of shrimp culture. WSSV infection in shrimp culture was triggered by fluctuations in temperatures of 3-4^{o}C, low salinity below 15 ppt, and high amounts of *Vibrio* [14].

Table 1. Prevalence Data of WSSV Presence on *Vannamei* Shrimp during the Study

Location	Total of Sample	Positive WSSV	Prevalence (%)
Bangil	20	2	15
Kraton	20	0	0
Rejoso	20	2	15

CONCLUSION

Based on the research that has been done in Bangil Sub-district, Kraton and Rejoso in Vaname Shrimp pons in Pasuruan, it is concluded that WSSV spread in Bangil and Rejoso Sub-districts during the research can be detected by positive samples marked by checking using Nested PCR with positive control used at 848 bp and 333 bp. Whereas the prevalence rate of WSSV attack on each region is as follows: Bangil 0 - 15%, Kraton 0% and Rejoso 0 - 15%.

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