Examination of White Spot Syndrome Virus (WSSV) in White Shrimp (*Litopenaeus vannamei*) and Tiger Prawn (*Penaeus monodon*) with Polymerase Chain Reaction (PCR) Method

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**Abstract.** Shrimp is one type of fishery commodity that can be processed into fishery products. Fishery products are included in the type of food that is susceptible to pathogenic microbial contamination. One type of pathogen that can contaminate the product the main fishery in shrimp is the White Spot Syndrome Virus (WSSV). The purpose of this study is to determine the technique of checking the WSSV virus on frozen products of white shrimp (*Litopenaeus vannamei*) and tiger prawns (*Penaeus monodon*). Research carried out in December 2019 – January 2020 at the Fish Quarantine, Quality Control and Safety of Fishery Products Surabaya II. The material used in the research is 3 samples of frozen tiger prawn product and 1 sample of frozen white shrimp product. Sample extracted using the Silica Extraction Kit and detected molecularly using the method conventional Polymerase Chain Reaction (PCR). This research is observational, then the data obtained were analyzed descriptively. The results showed that all samples shrimp negative for WSSV virus.

1. **Introduction**

Fisheries are one of the leading sectors that are in great demand by exporters, especially shrimp commodity. Shrimp commodity export value in 2017 reached US$ 1.7 billion with a percentage of 38.7%, making shrimp commodity a commodity superior product that occupies the first position as a fishery export commodity in Indonesia [1]. However, there are still several problems that become obstacles in meeting the high demand for shrimp products, one of which is the presence of a deadly virus in shrimp. A type of virus in shrimp that is quite dangerous, one of which is the White Spot Syndrome Virus (WSSV). Potential utilization of fishery products, especially shrimp in Indonesia the larger and more active the traffic of fishery products, both between countries and between areas within the territory of the Republic of Indonesia is an opportunity for an increased risk of entry and spread of pests and disease of fish both from within and outside the country.

WSSV virus has been found and attacks shrimp commodities in Indonesia and is included in the category Pests and Diseases Quarantine Fish (HPIK) group I. The presence of WSSV virus attack on shrimp commodities caused many quite detrimental impacts. Some of the impacts of the WSSV virus are the occurrence of mass deaths in shrimp farming, the rejection of shrimp commodities in export activities, thus causing a decrease in income. WSSV virus is microscopic and has a negative impact on the decline in the quality of fishery products, and can indirectly cause harm to humans because most types of viruses can be
zoonotic for humans. Although there is still no direct impact on human health, however WSSV virus attack can cause high mortality in shrimp [2].

WSSV virus examination on shrimp commodities is carried out as an effort to reduce the spread of the WSSV virus. Such testing must always be carried out regularly and strictly. One of the test methods that is widely used in the process of detecting the WSSV virus in shrimp commodities is using a Polymerase Chain Reaction (PCR) tool. A high level of accuracy and rapid detection is one of the advantages of using the PCR method in detecting types of microorganisms such as viruses [3]. Based on this background, it is necessary to make efforts to detect WSSV virus in shrimp products or commodities before distribution activities are carried out to reduce the level of spread of the WSSV virus by using the PCR test method.

2. Method

This research was conducted at the Molecular Biology Laboratory of the Fish Quarantine Center, Control Quality and Safety of Fishery Products Surabaya II from December 2019 to January 2020. The test method used in the WSSV virus detection process is use conventional PCR method. In general, the method of virus examination using conventional PCR method consists of the stages of sample necropsy, extraction, amplification, electrophoresis, and visualization results. It is important to sterilize equipment before carrying out the testing process. Sterilization process carried out by the wet sterilization method using an autoclave with a pressure of 1 atm at a temperature of 121°C for 15 minutes.

The organs taken in the necropsy process were the legs and tail of the shrimp. While the fry is taken whole the body due to its small size. The sample is inserted into the microtube as much as 20 mg for further extraction process. The extraction process is carried out to separate the acid nucleic acid from viruses with other cell components [4] by using the Silica Extraction Kit. Extraction the sample has three main stages, there are cell lysis process to remove viral DNA, extraction process to separate viral DNA or RNA content from cellulose and protein in cell, and DNA or RNA purification steps. The result of the extraction process in the form of supernatant was then put into a 500 μl microtube for further processing.

The next step that is quite important is the amplification process which functions to multiply the DNA of the tested virus [5]. The main components in the amplification stage used consist of DNA templates, primers, Taq DNA polymerase enzymes, deoxynucleoside triphosphates (dNTPs), magnesium chloride (MgCl₂), as well as PCR buffer solution. Stages of amplification consist of the pre-denaturation, denaturation, annealing, extension, and final extension. WSSV virus amplification process is carried out using the nested PCR method or two-stage amplification.

The samples obtained from the amplification results was then electrophoresed using a gel box. Sample is inserted into the hole of the agarose gel concentration 1.5% soaked with 1X TAE Buffer solution. The next gel box filled with viral DNA turned on by connecting the device to an electric current with a voltage of 115 volts for 45 minutes. The next step is to read the results using a UV transluminator. The results of the sample test will get a band line luminescence that can be observed on a computer that has been connected to the UV transluminator. The data from the results obtained were then analyzed descriptively qualitative, namely explaining in detail the results of the research obtained.

| Table 1. Composition of WSSV virus first PCR mix ingredients by IKM |
|-------------------------|-------------------|
| **Composition**         | **Volume**        |
| Master Mix              | 12.5 μl           |
| Primary 146F1 : 5’-ACT ACT AAC TTC AGC CTA TCT AG-3’ | 1 μl |
| Primary 146R1 : 5’-TAA TGC GGG TGT AAT GTT CTT ACG A-3’ | 1 μl |
| DNA Template            | 2 μl              |
| NFW                     | 8.5 μl            |
| Total                   | 25 μl             |
Table 2. First PCR amplification profile of WSSV virus by IKM

| First PCR WSSV Amplification Profile |
|-------------------------------------|
| Pre Heat                            |
| 94 °C, 4 minutes                    |
| 55 °C, 1 minutes                    |
| 72 °C, 2 minutes                    |
| 39 cycles                           |
| Denaturation                        |
| 94 °C, 60 second                    |
| Annealing                           |
| 55 °C, 60 second                    |
| Ekstension                          |
| 72 °C, 2 minutes                    |
| Final ekstension                    |
| 72 °C, 5 minutes                    |
| Hold / End                          |
| 4 °C, ∞                             |

Table 3. Composition of WSSV virus nested PCR mix ingredients by IKM

| Composition                     | Volume |
|---------------------------------|--------|
| Master Mix                      | 12.5 µl|
| Primary 146F2 : 5’-GTA ACT GCC CCT TTC ATC TCC A-3’ | 1 µl |
| Primary 146R2 : 5’-TAC GGC AGC AGC TGC TGC ACC TTG T-3’ | 1 µl |
| Amplicon (result of first step) | 2 µl   |
| NFW                             | 8.5 µl |
| Total                           | 25 µl  |

Table 4. Nested PCR amplification profile of WSSV virus by IKM

| Nested PCR WSSV Amplification Profile |
|---------------------------------------|
| Pre Heat                              |
| 94 °C, 4 minutes                      |
| 55 °C, 1 minutes                      |
| 72 °C, 2 minutes                      |
| 39 cycles                             |
| Denaturation                          |
| 94 °C, 60 second                      |
| Annealing                             |
| 55 °C, 60 second                      |
| Ekstension                            |
| 72 °C, 2 minutes                      |
| Final ekstension                      |
| 72 °C, 5 minutes                      |
| Hold / End                            |
| 4 °C, ∞                               |
3. **Results and Discussion**

Visualization results in the amplification process of WSSV virus examination on white shrimp and tiger prawns using the PCR method can be seen in Figure 1.

![Figure 1. Visualization of electrophoresis results (A. Tiger Prawn Sample; B. White Shrimp Sample) (Source: Documentation of Balai KIPM Surabaya II). Description: M = Marker; K(+) = positive control; K(–) = negative control; S(1) = sample DNA](image)

All samples that have been tested showed that the results were negative for the WSSV virus. The negative test results can be seen in the test sample holes that do not have a fluorescent line. While a positive test result for WSSV virus will show a fluorescent line with a size of 941 bp. [6]. The test sample will be declared positive for WSSV virus infection if the target DNA sequence can be detected amplified in the PCR method. Amplified viral DNA sequences in the process of electrophoresis will form a glowing band line.

Seen in the first hole, namely the M hole on the visualization of the electrophoresis results that is DNA marker that serves to determine the length of the amplified viral DNA fragment. The band lines on the DNA marker holes will be visible and glow at every 100 bp size. DNA markers that function to verify the size of the genotype of the virus will fluoresce well indicates that the PCR test process is running well without any contamination [7]. The second hole is positive control of WSSV virus that appears at a length of 941 bp. DNA band with 941 bp is the size of the WSSV virus band. Based on the results, the DNA band in the positive control WSSV appears and appears to glow clearly. The appearance of the band line of the positive control is used as an indicator of contamination or not, if the band line on the sample does not appear but the band line on the positive control appears, it can be concluded that there is no contamination [8]. The third hole is a negative control that does not have a band line, which is used to ensuring that no contamination occurs. If there is contamination in the test process, then the negative control may appear in fluorescent band lines. In the virus test process using PCR, Several parameters are needed to ensure that there is no contamination of the test results.

On the test results, it can be seen that there is a smear of luminescent band so that it can interfere with the process of reading the test results. The smear line on the PCR test results is indicated there is still protein and RNA content in the sample, thus interfering with the DNA detection process virus. The smear results can come from the remaining solutions that are still carried away during the extraction process or also derived from DNA that was degraded during the extraction process [9].
WSSV virus belongs to the type of double strand DNA (dsDNA) virus from the *Nimaviridea* family and the genus *Whispovirus*. The virions of the WSSV virus have an ovoid or ellipsoid shape to rod-shaped (bacilliform) with a length of 250-380 nm and a diameter of 80-120 nm. [10]. Clinical symptoms of infected shrimp WSSV virus is the presence of white patches on the body surface, empty intestine and pale body. The concentration of infected virus must reach 10-100 times the detection limit for WSSV to occur [11]. WSSV virus in shrimp infects at low and multiple levels. Low infection if the viral DNA only exists in the 296 bp luminescence band, which means 20 copies. Moderate infection in the 650 bp and 296 bp which means 200 copies. WSSV infection in severe cases has a luminescent band in 941, 650, and 296 bp which means 2000 copies. WSSV virus attack can cause financial loss large enough for shrimp farmers. Although there is still no direct impact on human health, but WSSV virus attack can cause high mortality rates in shrimp cultivation reached 100% [12].

4. Conclusion
The *White Spot Syndrome Virus* (WSSV) examination technique uses the PCR method on white shrimp and tiger prawns consist of several main stages, namely extraction, amplification, electrophoresis and diagnostic results. The results of the examination on 3 samples of tiger prawns and 1 sample of white shrimp showed that the shrimp samples were negatively infected by the WSSV virus. So, all samples tested are eligible for distribution process.

5. References
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