Examination of Taura Syndrome Virus (TSV) in white shrimp (*Litopenaeus vannamei*) and tiger prawn (*Penaeus monodon*) with Polymerase Chain Reaction (PCR) method

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Abstract. Frozen shrimp is one type of fishery product. Fishery products are a type of food that is susceptible to damage due to pathogenic microbial contamination. Virus is a type of pathogen that can attack fishery commodities including shrimp. One type of virus that attacks shrimp namely Taura Syndrome Virus (TSV). This study aims to detect Taura Syndrome Virus in frozen white shrimp (*Litopenaeus vannamei*) and tiger prawn (*Penaeus monodon*) products. The research was carried out in December 2019 - January 2020 at the Fish Quarantine Center, Quality Control and Safety of Fishery Products in Surabaya II. Materials used in this study consist of two types of frozen shrimp, namely 5 samples of tiger prawn and 2 samples of white shrimp. Virus testing samples were detected molecularly using the conventional Polymerase Chain Reaction (PCR) method which consisted of an extraction stage using a Silica Extraction Kit, an amplification stage, electrophoresis and result visualization. This research was observative, then the data obtained were analyzed descriptively. The results showed that all shrimp samples consisting of white shrimp dan tiger prawn were negative of the Taura Syndrome Virus (TSV).

1. Introduction
One type of fishery product that has a lot of export-import activities and is quite attractive in the market is frozen shrimp. Frozen shrimp in Indonesia is one of the leading export commodity product which have high value [1]. Every year, the demand for shrimp exports from Indonesia tends to increase, consisting of 80% frozen shrimp and 20% non-frozen shrimp [2]. However, one of things that causes shrimp production has not reached its maximum target is due to a disease caused by a virus. One type of virus that is quite dangerous for shrimp commodities namely Taura Syndrome Virus (TSV).

White shrimp and tiger prawns are among the types of shrimp that can host by TSV [3]. Classification of the Taura Syndrome Virus has been regulated in the Decree of the Minister of Marine Affairs and Fisheries Republic of Indonesia number 91/KEPMEN-KP/ 018 as Class I Quarantine Fish Pests and Diseases (HPIK).

Efforts to maintain the quality of fishery products can be carried out by means of testing on fishery products, especially virus inspection. This aims to prevent the spread of disease in shrimp caused by viruses as well as to monitor the quality of fishery products and ensure product safety [4]. TSV virus testing is usually done using the Polymerase Chain Reaction (PCR) method. The working principle of
PCR method is molecular examination by means of in vivo DNA amplification which is repeated throughout the reaction [5]. The purpose of this research is to detect the Taura Syndrome Virus on shrimp with using PCR (Polymerase Chain Reaction) method.

2. Material and methods
This research was implemented at Molecular Biology Laboratory of Fish Quarantine Center, Quality Control and Safety of Fishery Products Surabaya II on December 2019 to January 2020. Technique of examining Taura Syndrome virus on shrimp commodities was done using the conventional Polymerase Chain Reaction (PCR) method. There are 5 main stages in conventional PCR method testing process, namely necropsy the organ target, extraction with silica extraction kit, amplification using PCR machine, electrophoresis with agarose gel, and visualization the result with scan by computer. Virus testing using PCR method is carried out by paying attention to the level of sterility to avoid contamination in sample testing. One of the ways to minimize contamination is the equipment to be used using the wet sterilization method for 15 minutes with an autoclave of 1 atm pressure and with temperatures reaching 121°C.

Necropsy of samples is done by taking the target organ from TSV virus that is on gills, tail (uropod), body cuticles, and swimming feet. Meanwhile, for small shrimp or seeds is taken as a whole body. The sample used in the examination was taken as much as 20 mg and put into a microtube to do the extraction process.

| Table 1. Composition of TSV virus Single Step PCR mix ingredients by IKM |
| Composition | Volume |
|-------------|--------|
| Master Mix  | 12.5 µl |
| 9992F primer: 5' -AAG TAG ACA GCC GCG CTT-3' | 0.5 µl |
| Primary R3: 5' -TCA ATG AGA GCT TGG TCC-3' | 0.5 µl |
| AMV Reverse Transcriptase Enzyme | 0.5 µl |
| DNA Template | 1 µl |
| NFW | 10 µl |
| Total | 25 µl |

Process of sample extraction is carried out using Silica Extraction Kit. Cell wall of sample will undergo lysis in extraction process, so that the nuleic acid will be separated from other cell components [6]. Main stages on the process of DNA or RNA consist of extraction or separation of DNA or RNA from solid materials, and purification of DNA or RNA process [7]. 500 µl of supernatant extracted from sample was transferred into a new microtube for further amplification.

Amplification is main stage and most important process in PCR method. Amplification stage aims to multiply the target DNA to be tested [8]. The main components contained in the amplification stage is the DNA templates, primers, DNA Taq polymerase enzymes, deoxynucleoside triphosphate (dNTPs), magnesium chloride (MgCl₂) and PCR buffer solution. There are 5 stages in the amplification process, consisting of pre-denaturation, denaturation, annealing, extension and final extension. TSV amplification process is carried out using a single step PCR. Composition of TSV virus Single Step PCR mix ingredients by IKM (Table 1.).

Electrophoresis stage was carried out by inserting the amplified sample into the agarose gel hole which had a concentration of 1, 5% and then immersing it using a 1x TAE Buffer solution. Main working principle of electrophoresis process is migration of charged particles or molecules to electrodes that have opposite charges due to the influence of electric currents [9]. Electrophoresis process is carried out by connecting the gel box with an electric current with a voltage of 115 volts for 45 minutes (Table 2.). When UV light is turned on, the sample DNA band will appear and glow. Documentation of the sample results from electrophoresis process was carried out using a UV Transluminator that was connected to a camera and computer. Electrophoresis result will be displayed on the computer screen.
Data that has been obtained is then analyzed descriptively qualitatively by describing the results of the study using a series of words and compared using related references to explain and draw conclusions from the research results.

### Table 2. PCR amplification profile of TSV virus by IKM

| TSV Amplification Profile | Temperature, time |
|---------------------------|-------------------|
| Reserve Transcription     | 48 °C, 30 minutes |
| Pre Heat                  | 95 °C, 2 minutes  |
| 30 cycles                 |                   |
| Denaturation              | 94 °C, 45 seconds |
| Annealing                 | 60 °C, 45 seconds |
| Extension                 | 60 °C, 45 minutes |
| Final extension           | 72 °C, 45 minutes |
| Hold / End                | 4 °C, ∞           |

3. Results and discussion

Taura Syndrome Virus examination was carried out on 5 samples of tiger prawn and 2 samples of white shrimp. Visualization of the amplification results of TSV examination using PCR method on white shrimp and tiger prawns can be seen in Figure 1.

Based on the results of sample examinations using PCR method, all samples consisting of white shrimp and tiger prawn that were tested negative for Taura Syndrome Virus. Shrimp sample attacked by TSV are characterized by the appearance of a DNA band (band) at 231 bp in size [10]. DNA marker in hole 1 has a function to determine the size limit of the amplified DNA fragment. Based on the results, DNA marker bands appear on the electrophoresis result but looked thin. DNA marker line that appears can be an indicator that the electrophoresis process is running well [11]. Hole 2 is positive TSV control which has DNA band length at 231 bp indicates that the PCR process is running well. DNA band in TSV positive control based on the results appears clearly. The presence of positive control in this examination is used as an indicator if DNA band that appears in sample does not occur due to contamination or work errors [12]. The final result of the genomic band is influenced by the number of template DNA concentrations used in the electrophoresis process. Excessive amount of template DNA can cause the luminescence of the DNA band too thick, while too little amount of template DNA causes the luminescence of the thin DNA bands and even breaks [13]. Hole 3 is a negative control.
control that does not have DNA band, it serves to detect contamination in examination. Negative control in the PCR process serves to determine the possibility of contaminants that can interfere with the PCR process. The missing bands on the negative control indicate that the reaction mixture was not contaminated. Negative control presence of luminescence DNA bands was used as an indicator of contamination on PCR results [14].

Taura Syndrome Virus has an icosahedral shape with a diameter of 32 nm. The TSV virus genome contains 10.2 kb single strands and is included in the RNA virus group [15]. The clinical symptoms of shrimp infected with the TSV virus are reddish body color, especially on the tail and irregular black (melanizing) spots under the cuticle layer.

TSV examination on shrimp sample will be tested positive for pathogen infection if the target DNA sequence can be amplified by the PCR method. The amplified DNA sequence will be visualized and form DNA bands after electrophoresis using gel electrophoresis [16]. The appearance of the band on the positive control indicates that the PCR process is running well. Positive control serves to ensure that the PCR testing process runs according to specifications as well as a valid DNA sequence control in testing [17].

4. Conclusion

Taura Syndrome Virus (TSV) examination technique using the PCR method on frozen shrimp consists of several main stages, namely extraction, amplification, electrophoresis and result diagnosis. The results of the examination on five samples of tiger prawns and two samples of white shrimp showed that the shrimp samples were negatively infected by the TSV virus as indicated by the absence of the DNA band luminescence line at the size of 231 bp.

5. References

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