

## The Prevalence of White Spot Syndrome Virus on Tiger Shrimp (*Penaeus monodon*) Traditional Farming in Tarakan, Indonesia

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**ABSTRACT:** White spot syndrome virus (WSSV) is one of the main obstacles in the cultivation of tiger shrimp. Reports of WSSV infection in tiger shrimp traditional farming in Tarakan required for understanding on WSSV control. The paper was analysed 50 samples of tiger shrimps by using nested PCR method, and indicated four positive samples with WSSV molecular weight targets used 1447 bp and 941 bp. The prevalence of WSSV in January - March 2015 amounted to 8%. The results of this study showed that spreading of WSSV in tiger shrimp traditional farming in Tarakan allegedly caused by non-traceable sources of stem thus allowing the shrimps that infected WSSV transmitted from parents, environmental factors and lack of biosecurity applications. We argue that there were WSSV infections with a prevalence of 8% in various infections that showed by Nested PCR and clinical symptoms of infected samples.

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### INTRODUCTION

Tiger shrimp is one of the main export commodities of aquaculture for Indonesia. Tiger shrimp is a local species (endemic). Indonesian triumph as the country's producer and exporter of shrimp production decreased from 1995 were caused by several diseases such as WSSV. WSSV is one of the most dangerous pathogen in shrimp farming industry [1, 2]. A decrease in the production of tiger shrimp eventually many areas of Indonesia are turning to *vannamei* shrimp farming began in 2000. Tarakan region in North Kalimantan province is one area in Indonesia that still retains tiger shrimp traditional farming without feed and windmill also still continue to produce until now.

WSSV in tiger shrimp can occur at all levels of the stadia that can cause death to 100%. WSSV infection can occur vertically or horizontally. Control of the disease has become a major priority in tiger shrimp (*Penaeus monodon*) farming. According to Bachere [3], the durability of the shrimp production industry depends on a balance between the prevention of diseases with early diagnostic detection of diseases, assessment of health status and quality of the environment. In addition to detection of disease or survey aimed to estimate the prevalence of population disease status in a certain time [4].

The purpose of this study was to detect WSSV and prevalence in tiger shrimp traditional farming in Tarakan. In this study, Nested PCR used as a diagnostic method to detect the presence of WSSV in Tarakan. Nested PCR is a sensitive method that applied to determine the presence of WSSV were then used later to describe the prevalence of WSSV in Tarakan.

### MATERIAL AND METHODS

#### Samples of Shrimp

Tiger Shrimp samples used in this study came from traditional farming that do not use feed and windmills. Sampling was conducted in January - March 2015. 50 samples were taken from tiger shrimp traditional farming of the area in Tarakan Sampling was done randomly with no attention to the size and clinical symptoms are present.

#### DNA Extraction

Detection on WSSV in tiger shrimp conducted prior preparation of viral DNA through DNA extraction. Samples first homogenized by grinding entire organ until smooth, then extraction followed the manufacturer's procedures. DNA extraction in this study was using DNeasy Kit (Qiagen).

### Identification of Nested PCR

Examination by nested PCR was using two pairs of specific primers that 146F1, 5'-ACT-ACT-AAC-TTC-AGC-CTA-TCTAG-3', 146R1, 5'-TAA-TGC-GGG-TGT-AAT-GTT-CTT-ACG-A-3' and 146F2 (5'-GTA-ACTGCC-CCT-TCC-ATC-TCC-A-3'), 146R2 (5'-TAC-GGC-AGC-TGC-TGC-ACC-TTG-T-3'), the size aplikon 1447 bp and 941 bp [5].

### Prevalence of WSSV

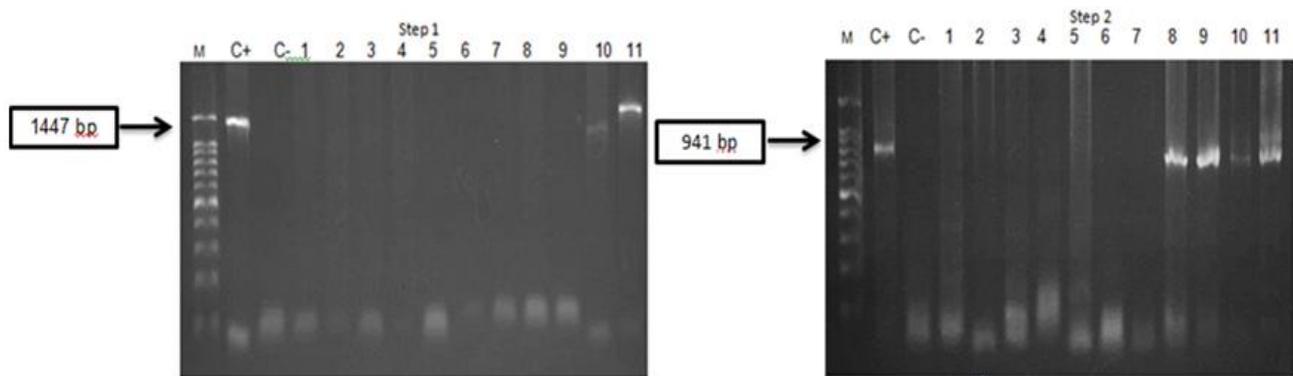
The estimation prevalence was calculated by using Natividad and Lightner method [6]

$$Prevalence = \frac{\text{Fish attacked}}{\text{Total of Population}} \times 100\%$$

## RESULTS

### Diagnosis of WSSV used by Nested PCR

By nested PCR test results indicated that the presence of WSSV attacks in tiger shrimp (Figure 1). It is characterized by the appearance of white stamp on the band 1447 bp and 941 bp which is the molecular weight of the DNA targets used WSSV virus.



**Figure 1.** Electrophoresis results control + at 1447 bp to 941 bp step 1 and step 2 for. (A). Line Samples 1-10 were amplified DNA samples of shrimp samples.

### Prevalence of WSSV

After PCR testing on 50 samples of tiger shrimp taken from some tiger shrimp traditional farming in tarakan region during the period in January - March 2015 obtained four positive samples infected with WSSV which 2 samples have WSSV clinical symptoms such as white spots (Figure 2) and 2 samples without clinical symptoms. Positive samples then calculated and the prevalence of WSSV was obtained by 8%.



**Figure 2.** Samples of infected shrimp WSSV (black arrows indicated the patches of white spot signs of WSSV clinical symptoms).

## DISCUSSION

Prevalence emergence of WSSV by using nested PCR was 8%. Of the 50 samples tested has found 4 positive samples WSSV infected. Positive samples infected with a molecular weight of 1447 bp DNA had clinical symptoms white spots on the body where white spot are specific lesion of WSSV [7]. White spot is irregularities of calcium metabolism where accumulate on the cuticle layer of shrimp [8]. Polymorphic white spots on shrimp WSSV signified the development of attack so that it can be used as an early diagnosis of WSSV [9]. WSSV attacked the target organ in ectodermal and mesodermal tissues (lymphoid, gut, gills, skin) while on the network endodermal attack hepatopancreas [7, 8]. In an attack against cells, using the WSSV virion protein coat [10]. On the positive WSSV infected samples on a molecular weight of 941 bp DNA did not revealed any clinical symptoms. This condition indicated the level of WSSV infection was still in mild scale. It is believed to be related also to the innate immune system which owned in haemolymph, it contained various types of hemocytes [11]. Shrimp infected by WSSV has a different resistance. Antiviral activity of hemocyanin is innate immune response against WSSV were able to delay viral infection and inhibit viral replication [12].

During the study did not found deaths on a large scale. WSSV infection found in Tiger shrimp traditional farming in Tarakan may be related to the source of the parent was not traceable therefore the fry were stocked in farming already was carrying WSSV transmitted vertically from parent [13] in addition to several other factors such as factor environment and lack of biosecurity application. Identification WSSV performed by Nested PCR greatly contribute to the prevention of WSSV through early detection of the diseases in presence, it is easier to control the disease.

## CONCLUSION

In the examination of tiger shrimp samples in traditional farming in Tarakan, North Kalimantan, has found WSSV infection with a prevalence of 8% with a rate of infection that is different where Nested PCR demonstrated through examination and clinical symptoms arising from any infected samples.

### Suggestions

Monitoring and inspection of WSSV in the fry will be stock on traditional farming in Tarakan can use Nested PCR because it has a high sensitivity in detection of WSSV. Research related to the spread of WSSV on traditional farming in Tarakan, North Kalimantan Province still needs to conduct so the pattern of WSSV spread in tiger shrimp traditional farming in Tarakan can be control.

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