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SURVEILLANCE OF WHITE SPOT SYNDROME VIRUS (WSSV) AND MYONECROSIS VIRUS (IMNV) INFECTIONS IN CULTURED *Litopenaeus vannamei*

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(Received 18 April 2018; Final revised 23 October 2018; Accepted 23 October 2018)

ABSTRACT

Disease surveillance programs will provide updated information on the distribution of diseases which allows proper development of mitigation and prevention strategies. WSSV and IMNV viruses are the most threatening diseases in shrimp and have affected the global shrimp farming industries. The present study was conducted to investigate the prevalence of WSSV and IMNV in three different locations of shrimp farms of South Sulawesi, West Nusa Tenggara and West Java. Samples of shrimp *Litopenaeus vannamei* were randomly collected from 20 active shrimp farms in the locations and five samples of postlarvae (PL) that were stocked in ponds. Tissue samples of the shrimp and whole body of PL were preserved in ethanol 90% for analysis. Determination of WSSV and IMNV were carried out by the mobile IQ™ WSSV and IQ™ IMNV Kit PCKIT Systems. The results showed that the prevalence of WSSV was 30% (6/20) and IMNV was 20% (4/20), while on PL was 40% (2/5). The result revealed that the South Sulawesi (Takalar) samples were positive only for WSSV while West Java (Panimbang) samples were positive only for IMNV. A follow-up study predicted that both viruses were transmitted from the PLs. The present study clearly confirmed that viruses were transmitted from PLs that were not free from either WSSV or IMNV. Therefore, a biosurveillance program is strongly recommended to be implemented in order to avoid the spread of the viral disease to other locations within Indonesia.

KEYWORDS: infectious; WSSV; IMNV; *Litopenaeus vannamei*; IQ™ Kit pockit

INTRODUCTION

Two important viral pathogens, white spot syndrome virus (WSSV) and myonecrosis virus (IMNV) have been reported in Indonesia (Senapin *et al.*, 2007; Taukhid & Nur'aini, 2009; Ferasyi *et al.*, 2015). Both virus diseases are the most important shrimp diseases in Indonesia. They affect most of commercially cultured Tiger and vannamei shrimps (Senapin *et al.*, 2011; Octovianus *et al.*, 2015). The first report of WSSV was in Taiwan 1992 (Chen, 1995) and was reported in China in 1993 by Huang *et al.* (1995) and spread worldwide ever since. The IMNV outbreak was first recorded in Brazil in South America (Lightner *et al.*, 2004), followed by Indonesia in Asia (Senapin *et al.*, 2007; Taukhid & Nur'aini, 2009). So far, there were

no reports about IMNV disease from other places found but as the seeds are procured from these countries. WSSV and IMNV have resulted in heavy mortalities and consequently production losses to the shrimp culture industry not only in Indonesia but also in many countries in Asia and Latin America (Senapin *et al.*, 2011; Arafani *et al.*, 2016). WSSV is extremely virulent with a wide host range of shrimp including *Penaeus monodon*, *Litopenaeus vannamei*, and *Penaeus stylirostris* and targets various tissues of ectodermal and mesodermal origin (Mahardika *et al.*, 2004; Dantas *et al.*, 2015). While IMNV mostly infects *L. vannamei* and may cause 100% mortality in case of a late handling and an early harvest. All sizes of shrimp can be infected by WSSV or IMNV in all kinds of aquaculture systems (extensive, semi-intensive and intensive and super intensive both larva and adult shrimp (Karunasagar *et al.*, 1997; Kilawati & Maimunah, 2015).

Disease surveillance plays an important role to predict, designate, and manage disease outbreak in shrimp culture. Active surveillance for potential viral

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pathogens is the core of mitigation measures in case of viral disease outbreaks. Surveillance ecosystems are established in order to substantiate the disease status of the area or even a country (OIE, 2010).

An excellent structured and planned strategy for all-around surveillance is necessary for a pilot-scale and long-term study in identifying and reporting pathogens. For this, cultured shrimps have to be tested at various levels at every stage of farming in order to identify any probable threats. A regular or periodic study in farms also is also necessary to trace pathogens that can survive long in the pond sediments and thereby provides ample time for planning remedial and management strategies. The present study was conducted to investigate the prevalence of WSSV and IMNV infections in three different locations of shrimp farms of South Sulawesi, West Nusa Tenggara and West Java. The results of the research are expected to serve as a reference in the prevention of viral diseases outbreaks. The present study also provides insights to the shrimp aquaculture industries to increase their awareness of the importance of disease surveillance and develop preventive strategies against viral diseases. Infectious of WSSV and IMNV are an Office International des Epizooties (OIE) listed penaeid shrimp disease (Flegel, 2006; Lightner, 2011; OIE, 2015).

MATERIAL AND METHODS

The first field survey involved a random collection of cultured shrimp from three farms at each different location i.e. South Sulawesi (Barru and Takalar Districts), West Nusa Tenggara (Sumbawa District) and West Java (Panimbang, Lebak District). The first field survey was carried out between April–July 2017 in active shrimp ponds. The second field survey also randomly collected samples of cultured shrimp at the same locations but in different shrimp farms that lasted from September to December 2017. In both field surveys, shrimp samples were also collected if there were clinical signs of diseases observed in the cultured shrimp and noted the duration of culture (DOC). Samples of postlarvae (PL) stocked in the ponds of Takalar and Panimbang were also collected in order to check any possible transmission of both viral diseases. Details of sampling locations, dates, sample codes, DOCs, tissues, and number of samples collected are presented in Table 1. A total of 25 samples (20 adults and 5 PLs) collected from the surveyed locations consisting of 335 individual shrimps were used in the virus detection analysis. The tissue samples of shrimp were firstly checked for visual clinical signs of virus infection following Nunan & Lightner (2011) description. Afterward, each 10 g of the tissue

samples of shrimp and the whole body of postlarvae (PL) was immersed in ethanol 90% before tested for WSSV using IQ Plus™ WSSV and RNALater® (Qiagen) before tested for IMNV using IMNV Kit POCKIT Systems. Both identification kit systems have been certified by the Office International des Epizooties (OIE).

Extraction of Nucleic Acid (DNA and RNA)

Extraction and amplification of Nucleic acids (DNA and RNA) for WSSV and IMNV detections followed the protocols described in the kits. All materials, solutions, and accessories were provided in the IQ Plus™ with POCKIT systems for the detection of WSSV and IMNV. The tissue samples of pleopods (50 mg) or 10 whole bodies of PL were used for the extraction. The tissues were placed in a 1.5 mL microtube and filled with 500 µL Solution-1 (lysis solution provided in the kit). A grinder was used to grind the sample directly in the tube. Afterward, The sample was mixed with 500 µL Solution-2 (with ethanol) and spun for 1 min. The produced supernatant was transferred to the spun column and the collection tube set and re-spun for 1 min. The solution was then discarded through flow-through from the collection tube whereas the supernatant was reassembled from the collection tube to the spun column. Of 500 µL of Solution-2 was added into the spun column and collection tube set, spin for 3 min., and discarded via the flow-through. The column was transferred to a fresh 1.5 mL centrifuge tube then added with 200 µL Solution-3 into the spun column and spun for 1 min. The nucleic acid (DNA/RNA) was diluted into the tube and ready to be used for the amplification process.

Amplification

The IQ Plus™ POCKIT for WSSV and IMNV detection came with a ready Premix Pack. The first detection step was preparing the required vials according to the number of samples to be analyzed. Each vial cup was added with 50 µL Premix buffer B to re-suspend the mix pellets (containing dNTPs, specific primers, fluorescent probes, and enzyme). Samples of the previously prepared DNA/RNA extract samples and positive control were separately collected and dissolved into different premix tubes using inoculation loops, mixed well, and spun slowly. The sample and positive control were transferred into the R-tubes, placed in a holder, and spun for 10 seconds. The POCKIT™ Nucleic Acid Analyzer was turned on and completed a self-testing for 5 min. The marked wavelengths of 520 nm + 550 nm were selected for further analysis as mentioned in the manual. After the system ready, the result was displayed and the holder containing the R-tubes was placed into the reaction

Table 1. Samples of *L. vannamei* for WSSV and IMNV analyses collected from different farm locations

Location	Date of sampling 2017	Sample code	Days of culture (DOC) when sampling	Tissue collected	Number of sample (ind.)	
South Sulawesi	Barru	BAA-1	86	Gill and pleopod	10	
		BAA-2	87	Gill and pleopod	10	
	Takalar	April, 24	TASY-1	25	Gill and pleopod	15
		April, 25	TAM -1	31	Gill and pleopod	10
		April, 25	TASY-2	31	Gill and pleopod	15
NTB	Sumbawa	SJHR-1	88	Gill and pleopod	10	
		SJSB-1	79	Gill and pleopod	10	
West Java	Panimbang	April, 28	PAH-B	PL-12	Whole	20
	Panimbang	July, 1	PJ A-1	93	Gill and pleopod	20
			PJB-2	95	Gill and pleopod	20
		July, 3	PJD-1	95	Gill and pleopod	20
	PJD-3		94	Gill and pleopod	20	
South Sulawesi	Takalar	September, 20	TSPL-1	PL-10	Whole	15
			TSPL2	PL-10	Whole	15
	Takalar	November, 17	TNSY-1	58	Gill and pleopod	10
		November, 18	TNSY-2	59	Gill and pleopod	10
		November, 18	TNSY-3	63	Gill and pleopod	10
NTB	Sumbawa	November, 27	SNUR-1	78	Gill and pleopod	10
		November, 28	SNUR-2	90	Gill and pleopod	10
West Java	Panimbang	October, 6	POSMI-1	PL-9	Whole	15
			POSMI-2	PL-9	Whole	20
	Panimbang	December, 12	PDSMI-D1	56	Gill and pleopod	10
			PDSMI-D3	56	Gill and pleopod	10
			PDSMI-A2	60	Gill and pleopod	10
			PDSMI-B1	60	Gill and pleopod	10

chamber of POKKIT™ Nucleic Acid Analyzer which was run to begin the reaction program. The test result was shown on the screen after the reaction. The cleared protocol of analysis can be found in the operational procedure of The IQ Plus™ POKKIT System.

RESULTS AND DISCUSSION

All shrimp samples obtained from the farms and postlarvae (PLs) were analyzed by the IQ Plus™ POKKIT (Figure 1 and 2). The line marked with ? (Question) means that DNA or RNA virions were less than 10 copies and have to be re-analyzed (Figure 1 and 2). After re-analyzing, the samples were confirmed positive for WSSV and negative for IMNV. The bottom lines marked with positive signs both for WSSV and IMNV indicated good internal control for analyzing

process (Figure 1 and 2). From the 20 shrimp farms and 5 PL hatcheries that were randomly selected for this study, six shrimp farms were infected by WSSV and four farms by IMNV. Based on the information from the 20 farm owners, ten farms had a successful harvest, and the rest failed in different stage/days of culture (DOC) (Table 2).

A follow-up study was conducted in shrimp farms stocked with PLs positively tested for WSSV or IMNV by the IQ Plus™ POKKIT. The successful harvest in shrimp farms stocked with tested PLs virus-free proves that the IQ Plus™ POKKIT system can be considered a good diagnostic tool and can be employed for virus screening in PL and broodstock as suggested by Koesharyani & Gardenia (2015). The results showed that the prevalence of WSSV was 30% (6/20) and IMNV

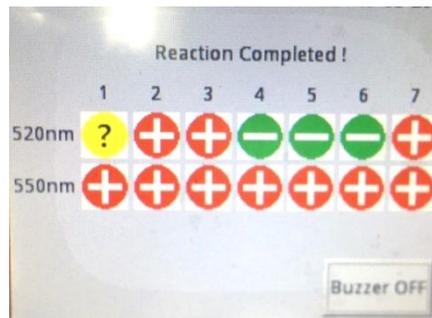


Figure 1. The IQ Plus™ POCKIT for WSSV detection products from representative samples (Top Line) with WSSV-positive lines 2 and 3, WSSV negative lines 4-6, and control line 7. Marked ? in line 1 was DNA virion which was less than 10 copies and was re-analyzed and confirmed positive for WSSV, while bottom line indicates good internal control.

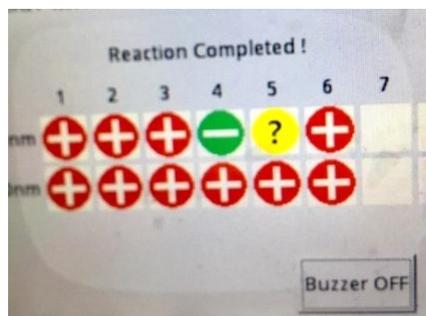


Figure 2. The IQ Plus™ POCKIT for IMNV detection products from representative samples (Top Line) with IMNV positive lines 1 to 3, IMNV negative lines 4, and line 5 marked ? was DNA concentration which was less than 10 copies and was re-analyzed and confirmed negative, and positive control line 6, while bottom line indicates good internal control.

was 20% (4/20) while in PL's was 40% (2/5) (Table 2). Interesting results revealed that WSSV positively infected the shrimp farms in Takalar while IMNV infected the shrimp farms only in Panimbang. The occurrences of WSSV and IMNV infection found in this study could be attributed to the contaminated PLs as showed in Table 2. WSSV is extremely virulent with a wide host range and targets various tissues of ectodermal and mesodermal origin (Prasad *et al.*, 2017). This study suggests that all age groups and sizes of shrimp are affected by WSSV or IMNV in all kinds of aquaculture systems (extensive, semi-intensive, intensive, and super intensive). In this study, the shrimp samples from Takalar were collected from intensive and super intensive farms with stocking density between 150-750 ind./m³, while samples from Panimbang and Sumbawa were collected from extensive, semi-intensive, and extensive farms with stocking densi-

ties between 60-150 ind./m³. Transmissions of WSSV and IMNV occurred vertically from the infected broodstock to larvae or horizontally through water or infected animals (Hossain *et al.*, 2001; Otta *et al.*, 2003). In the present study, positively tested WSSV or IMNV PL's stocked in farms had resulted in emergency harvest, while farms stocked with negative WSSV or IMNV had a normal harvest. However, some previously tested virus-free farms carried out emergency harvest that this study suggests a possible virus transmission at the same location via water from infected farms (Table 2).

Presently, WSSV is prevalent all over Asian countries (Corteel, 2013) while IMNV is prevalent only in Indonesia despite shrimp farming has been actively practiced in the nearby countries (Senapin *et al.*, 2007; Tauhid & Nur'aini, 2009). Indonesian shrimp farming practices use a higher rate of stocking densities

Table 2. Screening results of shrimp (*Litopenaeus vannamei*) samples collected from different locations for WSSV and IMNV infections using IQ Plus™ POCKIT

Locations	Date of Sampling 2017	Sample Code	Life stage collected (Days of culture /DOC)	No. of sample (Ind.)	Status of virus	
					WSSV	IMNV
Barru	April. 24	BAA-1	86	10	-	-
	April. 24	BAA-2	87	10	-	-
Takalar	April. 25	TASY-1	25	15	+	-
	April. 25	TAM -1	31	10	+	-
	April. 25	TASY-2	31	15	+	-
Sumbawa	June. 4	SJHR-1	88	10	-	-
	June. 4	SJSB-1	79	10	-	-
Panimbang	April. 28	PAH-B	PL 12	20	-	-
Panimbang	July.1	PJ A-1	93	20	-	-
	July.1	PJB-2	95	20	-	-
	July.3	PJD-1	95	20	-	-
	July. 3	PJD-3	94	20	-	-
Takalar	Sep. 20	TSPL-1	PL10	15	+	-
	Sep. 20	TSPL2	PL10	15	+	-
Takalar	Nov. 17	TNSY-1	58	10	+	-
	Nov. 18	TNSY-2	59	10	+	-
	Nov.18	TNSY-3	63	10	+	-
Sumbawa	Nov. 27	SNUR-1	78	10	-	-
	Nov. 28	SNUR-2	77	10	-	-
Panimbang	Oct. 6	POSMI-1	PL-9	15	-	+
	Oct. 6	POSMI-2	PL-9	20	-	-
Panimbang	Dec. 12	PDSMI-D1	56	10	-	+
	Dec. 12	PDSMI-D3	56	10	-	+
	Dec. 12	PDSMI-A2	60	10	-	+
	Dec. 12	PDSMI-B1	60	10	-	-

in the range of 60 to 1,000 ind./m³ (Adiwijaya *et al.*, 2008; Atjo, personal communication). High stocking density leads to increase chances of contact between the cultured individual and also escalates the probability of ingestion of WSSV and IMNV contaminated tissues (Graf *et al.*, 2004). The onset of the disease is related to stress factors ranging from changes in salinity and temperature to rough handling during partial harvest or sampling by cast net. Temperature also plays a crucial role in the occurrence of diseases. Exposure to an elevated temperature could lead to excessive feeding which subsequently leads to elevated ammonia concentrations in the culture system (OIE, 2012). Indonesian climatic conditions like high atmospheric temperature can accelerate the stress conditions of shrimps thereby rendering them susceptible to WSSV or IMNV infection (Arafani *et al.*, 2016). The present study demonstrated that the seeds stocked in Takalar or Panimbang shrimp farms were not free from WSSV and IMNV (Table 2). This was the main cause of disease outbreaks of WSSV and IMNV in these locations. The study also demonstrated that Indonesian shrimp farms located along the coastal areas are not free from WSSV or IMNV. This study is arguably the first report on shrimp DNA and RNA risk-

based targeted disease surveillance of the viruses, at least for Indonesia. Based on the field observation, the PLs used in the farms were non-traceable. Some of the farms did not have proper biosecurity management with a poor environmental condition which increases the risks of infection of WSSV and IMNV. Therefore, a biosurveillance program is strongly recommended to be implemented by the government to avoid the spread of the viral diseases in other locations within the Indonesian region. Hatcheries producing PL should be requested to make sure that the PLs are free from the viruses and only PLs that are certified pathogen free (SPF) are allowed to be sold to farmers.

CONCLUSION

The prevalence of WSSV was 30% (6/20) and IMNV was 20% (4/20), while on PL was 40% (2/5). In South Sulawesi (Takalar), shrimp samples were positive only for WSSV while West Java (Panimbang) samples were positive only for IMNV. Based on the research findings, this study recommends that a biosurveillance program has to be implemented in order to avoid the spread of the viral diseases to other locations within Indonesia.

ACKNOWLEDGMENTS

The author thank the representative technicians, Mr. Saiful from Takalar farms, Mr. Mulyadi from Panimbang farms, and Mr. Imron from Sumbawa farms for their help in collecting samples and share of information regarding their work experience in shrimp farms. This study was supported by the APBN 2017 annual budget through the Research Center for Fisheries.

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