Molecular Detection of White Spot Syndrome Virus (WSSV) and Taura Syndrome Virus (TSV) on Vaname Shrimp (*Littopenaues Vannamei*) Using Polymerase Chain Reaction (PCR) Method at BLUPPB Karawang

Nur Ikhsan Ma'arif*, Rapa Bagus Panuntun, Silviana AlyaniAzy, Ayyash Eundang Nurul Haqi, Nasywa Nur Fauziyah, Shobrina Silmi Qori tartila

Department of Aquaculture, Faculty of Agriculture, Universitas Tidar
Jl. Kapten Suparman No 39, Magelang Utara, Magelang, Jawa Tengah , Indonesia
***Correspondence** : ikhsanmaarif09@gmail.com, Phone 085892087574

.

ABSTRACT

Shrimp production numbers can decrease due to diseases caused viruses, bacteria or parasites. The purpose of this research is to find out the mechanism and find obstacles in detecting TSV and WSSV diseases using the PCR method. The research has been carried out in the time period January 3 - February 3, 2024 which is located at BLUPPB Karawang. The number of samples was 15 shrimps which were selected randomly and divided into 3 groups, each containing 5 shrimps (codes W.01; W.02; and T.01). The data analysis method uses qualitative data analysis by examining the results of observations in visual form. The results showed that sample W.01 formed DNA bands between 630, 550, and 333 bp, sample W.02 formed bands between 630 and 550 bp, while sample T.01 formed RNA bands only at 476 bp. Efforts to implement biosecurity is a method that can be applied to prevent infection rates because no cure has been found for WSSV and TSV viruses that infect shrimp.

Keywords: *BLUPPB Karawang, PCR, TSV, Vaname shrimp, WSSV.*

INTRODUCTION

Vaname shrimp is one of the commodities in the fisheries sector that plays a role in fulfilling food needs in Indonesia. Based on data reported from KKP statistics (2022) the level of shrimp production in Indonesia reached 71,911,555.00 million tonnes. Shrimp production figures can decrease due to disease in shrimp. Shrimp disease is a disease suffered by shrimp due to exposure to viruses, bacteria or parasites that can reduce production value because it reduces the growth rate and even causes death in shrimp. Quoting from some literature, examples of viral diseases that infect shrimp are as follows. WSSV was first reported to infect vaname shrimp in shrimp ponds in China and Taiwan in 1992, this virus has a mortality rate of 5 - 90% but does not always lead to death in shrimp. TSV was first reported in Ecuador in 1992 in the juvenile phase of vaname shrimp, TSV mortality rates reached 60-90%.

Efforts that can be made to anticipate the number of viral disease spread and failure during the cultivation process can be done through integrated *control* and monitoring of pathogenicity levels in the cultivation environment during the production period. The process of detecting pathogens can be done using *Polymerase Chain Reaction* (PCR) techniques that function to detect molecular viruses that attack vaname shrimp.

The application and molecular testing of diseases in aquaculture is minimal due to the high cost of testing infected samples. According to molecular testing to monitor the infection status of aquatic animals against diseases is rare compared to terrestrial animals. Health certification based on OIE guidelines for testing against viral diseases currently

places more emphasis on the use of molecular methods due to the difficulty of validating serological methods. In Indonesia, molecular testing to determine the level of disease infection in aquaculture is only carried out at large fisheries centres that have adequate facilities and not all fisheries centres have PCR testing.

Based on this statement, the aim of research was to detect prepared Molecular Detection of *White Spot Syndrome Virus* (WSSV) and *Taura Syndrome Virus* (TSV) in Vaname Shrimp (*Litopenaeus Vannamei*) Using the *Polymerase Chain Reaction* (PCR) Method at the Karawang Fisheries Production Business Service Center (BLUPPB).

MATERIAL AND METHODS

Primary data collection by means of observation, PCR testing, and conducting interviews with experts at BLUPPB Karawang. The data analysis used in this test is a qualitative descriptive method, the method describes and provides an overview of the phenomena studied systematically and realistically based on the research results. In addition, data analysis was carried out using several relevant literature sources.

IQ2000^{TM}(2018) explains that the appearance of one DNA band indicates mild infection (I), the formation of two DNA bands indicates moderate infection (II), and if three DNA bands are formed, it indicates severe infection (III), if only at 848 bp, it indicates that the genome was successfully extracted (*housekeeping gene*) or indicates that the absence of bands that appear indicates negative WSSV. According to IQ2000^{TM} (2018), TSV samples are said to be positive if DNA bands are formed at positions 284 or 476 bp.

DNA and RNA Extraction

The sample extraction stage begins with taking 15 shrimp from the enlargement pond with a size of 7-14 cm, which are then brought to the fish health laboratory for several stages of TSV and WSSV testing. Samples were randomly selected to be divided into 3 sub-samples, each of which contained 5 shrimp. 1 DNA extraction sample contains 5 shrimp containing the target organ of each virus. The advantage of extraction using the silica method is that the final sample can be used for testing of DNA and RNA.

Extraction was carried out using the IQ real extraction method in the form of silica gel. The organs taken in this method are swimming leg, walking leg and gill samples. Samples that have been prepared are taken from the target organ using a *dissecting set* and then weighed as much as 20 mg and put into a 1.5 ml *microtube*. The second stage added 900 µl of GT Buffer solution and mashed with a grinder. The third stage After the sample has a smooth level, the next stage is carried out centrifuge for 3 minutes at 12000 rpm and transferred the top of the solution as much as 600 µl into a new *microtube*. The fourth stage The sample that has been transferred to a new tube is then added with 40 µl of silica gel solution and homogenised again at 12000 speed for 15 seconds. The fifth stage of the supernatant part of the sample was discarded and washed again with 75% ethanol as much as 1 ml and centrifuged again for 15 seconds at 12000 rpm. In the extraction stage, the washing process has a function to break down DNA and RNA particles so that the cells are perfectly purified. The sixth stage adds DEPC $_{ddH2}O$ as much as 1 ml into the pellet and then homogenised, after which incubation is carried out at 55ºC for 10 minutes and centrifuged for 2 minutes at the same speed as the previous stage. The last step is to transfer the top solution as much as 500 µl into a new tube. Samples that have passed

the extraction stage can be tested at the next stage.

DNA and RNA Amplification

Amplification of WSSV was carried out after sample extraction. Primer sequences used in the first PCR of WSSV were Premix 146F1, (5'-ACT-ACT-AAC-TTC-AGC-CTA TCT AG-3') and 146R1, (5'-TAA-TGC-GGG-TGT-AAT-GTT-CTT-ACG-A-3'). Amplification is done by taking 2 µl of DNA and inserted in each tube (Positive Control, Negative Control, and main sample) after that it is done by making a mixture for mixing Reagent first PCR as much as 7.5µl and Iqzyme DNA Polymerase 0.5µl then input to each sample. In the positive control sample, 2µl of P (+) Standard was added and 2µl of Yeast tRNA was added to the negative control. If the sample has been done the amplification is done using a thermal cycle with a temperature of 42ºC x 30 minutes, 94ºC for 2 minutes (*denaturation)*, 94ºC x 20 seconds, 62ºC x 20 seconds, 72ºC x 30 seconds for 15 cycles (*annealing)*, and 72ºC x 30 seconds, 20ºC x 30 seconds (*extention)*.

After the first stage amplification is complete, the nested stage is carried out with primers such as 146F2 (5'-GTA-ACT-GCC-CCT-TCC-ATC-TCC-A-3') and 146R2 (5'-TAC-GGC-AGC-TGC-TGC-ACC-TTG-T-3'). addition of reagents in the form of Nested PCR Premix 14 µl and Iqzyme DNA Polymerase 1 µl, after which it was put back into the *thermal cycle* with a temperature of 94ºC x 2 minutes, 94ºC x 20 seconds, 62ºC x 20 seconds, 72ºC x 30 seconds for 30 cycles (*annealing)*, and 72ºC x 30 seconds, 20ºC x 30 seconds (*extention*).

The amplification process in TSV has a slight difference with WSSV because the genetic material of TSV is RNA. The amplification process primers used were 1004F (5'-TTG - GGC - ACC - CGA - CAT T-3') and 1075R (5'-GGG - AGC - TTA - AAC - TGG - ACA - CAC - TGT-3'). The first stage is *mixing* RT-PCR Premix 7.0 µl, Iqzyme DNA Polymerase 0.5 µl, and RT Enzyme 0.5 µl. After that, it was entered into a thermal cycle with a temperature of 42ºC x 30 minutes and 94ºC x 2 minutes (*denaturation*). 94ºC x 20 seconds, 62ºC x 20 seconds, 72ºC x 30 seconds is done for 15 cycles (*annealing*) and 72ºC x 30 seconds and 20ºC for 30 seconds (*extention*). After completion of the Nested PCR stage by mixing 14 µl Nested PCR reagent and 1 µl Iqzyme DNA polymerase and then re-entered the thermal cycle with *annealing* temperature 94ºC x 20 seconds, 62ºC x 20 seconds, 72ºC x 30 seconds performed as many as 30 cycles and *extention* 72ºC x 30 seconds and 20ºC for 30 seconds. After amplification of both samples, the next step is electrophoresis.

Electrophoresis

Before the electrophoresis process, there is a stage of making 2% agarose gel. Agarose gel is a material that has a density resembling agar which has a function to separate DNA or RNA particles towards the electric current charge. The first stage of making agarose gel is done by weighing agarose powder using analytical scales as much as 1g. The second stage is to do a 50 x dilution of TAE so that 20 ml of TAE and 980 ml of distilled water are obtained. The third stage is the TAE solution is homogenised with agarose powder until it dissolves. The fourth stage of the solution is then cooked using an electric stove, the maturity parameter can be seen if there is a change in colour to clear. The last stage is adding 1 µl of gel red and then printed using electrophoresis agar moulds.

The solidified agarose gel was soaked with TAE (*Tris-acetate-EDTA*) solution and inserted into a horizontal electrophoresis machine. The first stage in electrophoresis is the sample that has been

amplified added 6x *loading dye* as much as 5 µl and then homogenised, it has a function to help DNA or RNA in moving during the electrophoresis process. The second stage, the wells formed are inserted DNA Marker with the position of the initial wells, positive control samples in the second well, negative control in the third well, the main sample in the fourth well, and duplo samples in the fifth well. The electrophoresis process runs approximately 20-30 minutes.

Visualisation of results

The agarose gel that has been electrophoresed is then taken and inserted into the UV transilluminator. After that, the tool was turned on until purplish-coloured UV light was seen. Observations were made on the outside glass and documentation was done using a camera. The success parameter of PCR is characterised by the formation of DNA bands in the wells.

RESULT

The results of WSSV and TSV testing using PCR showed the test results listed in Table 1.

No	Sample code	Organ infected with virus	Vaname shrimp code	PCR tes result
1.	W.01	Swimming legs And walking legs.	• Medium body size • White spot in carapas • Slightly reddish body	Positive
2.	W.02	Swimming legs And walking legs.	• Medium body size • White spot in carapas • The body is slightly reddishin color	Positive
3.	T.01	Swimming legs And walking legs.	• Medium body size • White spot in carapas • There is a color change to reddish on the tail	Positive

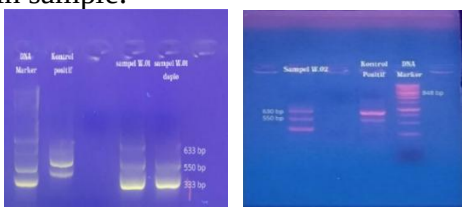
DISCUSSION

Sample results are said to be positive if they are found in a straight line with the positive control and DNA Marker, and negative controls that are visualised do not appear in the agarose gel. This is in line with the statement that the indicator of a positive sample is that the positive control is in line with the DNA Marker. Marker DNA has a function as a reference control to determine the value of basepare (bp), this is because marker DNA has a genomic property, so that sequence analysis can be carried out on the organism being tested. Visualisation of the results of the virus infection level was analysed through the brightness level of the virus basepare. According to the level of infection, the visualisation results are divided into several groups, namely; light, very light, medium, and serve.

White Spot Syndrome Virus (WSSV)

Electrophoresis visualisation using a UV transilluminator showed that both samples tested were indicated to be infected with WSSV virus, according to Figure 1, the negative control did not appear on the screen, and the samples tested appeared in

a straight line with the DNA Marker and positive control. The diagnostics performed found that sample W.01 contained a heavy positive result and formed three DNA bands between 633, 550 and 333 bp with a bright brightness level (*serve*), indicating that the sample indicated heavy infection. Samples infected with WSSV that have severe symptoms have the formation of three DNA bands which means that the virus has replicated as many as 2000 copies. Sample W.02 has a positive result with DNA bands formed between 630 and 550 bp. According to , the positive WSSV results formed in the two DNA bands showed *amedium* positive level, explaining that the type II (*medium*) infection level shows clinical symptoms in the form of reddish colour on the body surface and begins to enter the acute infection phase. This infection process is thought to be completed in a very short time due to the high viral load and small host size, and therefore white spots do not appear on the body surface. The duplo sample placed next to the main sample in PCR testing has a function as a harmoniser in the PCR stage, so that the results obtained are similar to the main sample.



Sample (A). W.01. (B). W.02.

The initial stage of the WSSV virus process is to find a suitable host to enter the replication stage. This is followed by absorption on the host cell surface and attachment of proteins located on the capsid (Stansfield, *et al*., 2003). The WSSV attachment process begins with the attachment of ionic levels which is done by shifting pH levels or salt concentrations. The next stage is the attachment of viral proteins with specific polysaccharides on the host surface by viral protein - 28 which has the function of binding to receptors on the host. Viral Protein-28 (VP28) is an adhesion protein that helps the virus to bind to shrimp cells and enter the cytoplasm during infection, and can bind to actin or actin-related proteins and help WSSV translocate to the nucleus. explained that the ion shift process carried out by WSSV causes an increase in the stress response in shrimp. In addition, cultivation environmental factors such as temperature, salinity, pH, dissolved oxygen, ammonia fluctuations can affect the stress response, so that it can interfere with the immune ability and metabolic performance of shrimp, thereby increasing susceptibility to microbial infections. These extrinsic factors also have a level of influence on the biochemical processes that occur in shrimp so that they can cause reduced metabolic processes in haemolymph in the form of protein, glucose, triacylglycerol, and others.

Transmission of WSSV virus can occur through various aquatic organisms in culture ponds as well as those from outside the culture ponds. Aquatic and benthic organisms such as polychaeta worms, microalgae, rotifer eggs, and seagulls are known to be carriers of WSSV. Other things that can support the pathogenicity of WSSV can occur through horizonal changes through the water and sediment contained in the pond. The organs that were examined for White Spot Syndrome Virus (WSSV) were swimming legs and walking legs which were taken as much as 20 mg. Swimming legs are one of the organs that are susceptible to viral infection, WSSV virus infection can occur through soil sediments that are infective to shrimp so that if shrimp do activities on the soil sediments it can allow infection caused by WSSV.

The hepatopancreas is an organ that has a function to absorb nutrients in digested feed, produce organic and mineral substances and metabolise organic and inorganic compounds that have a role in digestion. The hepatopancreas is one of the organs that is susceptible to WSSV infection through oral entry due to contaminated water media. WSSV can also infect the hepatopancreas, mainly confined to myoepithelial cells of the hepatopancreatic sheath and fibroblasts of the connective tissue of the tubular epithelial tissue. The impact of WSSV infection on the hepatopancreas is decreased organ function, slowed metabolism and cell decay. This is in line with Lightner's (1996) statement that hepatopancreas infected by WSSV can cause organ dysfunction, slowed growth, cell decay and death in a short time.

Hypoxia in shrimp is a condition of lack of oxygen that occurs in shrimp due to decreased water quality, increased residue in the pond body, to diseases that infect the respiration organs of shrimp. explained that viral infections can occur through the oral route or through water to the gills or cuticular epidermis. Infection of the gills can cause cell damage so that shrimp will have difficulty in taking oxygen, this can have an impact on shrimp metabolism which slows down and changes in behaviour such as swimming obliquely and shrimp often come to the surface to take oxygen.

Biosecurity measures that can be taken for the prevention of WSSV are post-harvest draining of culture ponds, low water exchange systems such as RAS, limiting access to vectors and pathogens (through crab fences, bird barriers, and foot baths on shrimp farm entrances), and increasing disease resistance (immunostimulants, neutralisation, environmental management and vaccines) in shrimp. This is an effective management method, as there is still no treatment for WSSV infection.

Taura Syndrome Virus (TSV)

Taura syndome virus is a virus that has genetic material in the form of RNA and has a single strand that infects vaname shrimp. TSV is a virus that belongs to the family Dicistroviridae, this virus has a positive single-chain RNA that has an icosahedral shape and has a genome of 10.2 kb with a diameter of 32 nm. Based on the electrophoresis results in Figure 7 using 5 shrimp samples and visualisation results using UV transilluminator, positive results were obtained with the position of the viral RNA band formed between 476 bp. The results show that the positive control band is formed but the level of its appearance is not clearly visible, this can be caused because the positive control used is old and starting to break down. Similar to the marker statement that is not clearly visible can be caused by poor marker conditions.

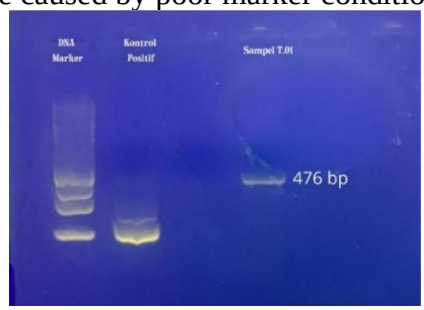


Figure 2. Sample T.01

Organs used in testing were swimming legs, walking legs, and gills because they are the main targets of WSSV infection. According to TSV virus testing is conducted using PCR assays, such as commercial RT-PCR kits and reverse transcriptase PCR (RT-PCR) using TSV virus target organs such as uropods, gills, body cuticles, and swimming legs. Another similar opinion was expressed by the OIE (2018), which stated that haemolymph, pleopod and gill samples can be used as extraction samples in PCR testing.

TSV has 3 stages of infection process, namely transitional, chronic, and acute stages. The transitional stage of infection is characterised by melanisation of the chepalothorax and tail with behavioural symptoms such as shrimp lethargy and anorexia. Histological description of TSV-infected shrimp in the transitional stage of infection shows that TSV infection begins within 24 hours of natural or experimental survival of individuals with acute infection will experience multifocal melanised lesions, and anomalies in lymphoid organs and arterioles. The chronic stage of TSV infection can be prevented from spreading using the humural immune system in shrimp. According to the chronic stage of TSV infection, there are no symptoms, but histologically there are many lymphoid organs. The apoptotic immune system plays its role at this stage of infection by preventing the replication of the virus and eliminating cells that infect the host. The acute stage of TSV infection begins to show symptoms of physical changes in the infected host. According to the organs, the gills, intestines and culticula of the body begin to be infected with physical symptoms of black discolouration of the body surface, reddening of the tail and softening of the shell.

TSV is highly pathogenic even in dead shrimp. This virus can survive for up to 3 weeks. Research conducted by showed that TSV can be a further infectious agent in shrimp that have experienced a 48-hour mortality period and can survive for up to 3 weeks. Transmission that can occur is through oral or when other shrimp consume TSV-infected shrimp so that it can cause a continuous chain of infection. External factors that can cause TSV infection are organisms from outside the pond such as seagulls, annelids, and periphyton such as aquatic insects that live on the pond surface. The use of drugs for the prevention and treatment of infections caused by TSV has not yet been found. Prevention stages that can be done in the form of implementing a biosecurity system such as the selection of pathogen-free seeds, bird barriers and closing the inlet channel with nets aimed at preventing other organisms from entering the pond, as well as feed rich in nutrients to increase immunity in shrimp. According to the things that can be done to prevent TSV infection are regulating the water management cycle, sanitising the cultivation environment, using pathogen-free broodstock and seeds, and screening using biotechnology such as PCR and ELISA.

Obstacles in testing

A common obstacle encountered during the detection of WSSV and TSV using PCR is contamination of the negative control resulting in a positive band. Contamination that occurs can come from a contaminated external environment, contamination of the tools used, to contaminated reagent samples. Efforts for this problem are to clean laboratory equipment and workspace with disinfectants and perform physical sterilisation using UV light. If reagent contamination occurs, it must be replaced with a new one so that further contamination does not occur in testing other samples.

Another technical problem that occurred was that the marker band appeared but there was no positive control. This is due to the process of PCR stages such as temperature errors and pipetting techniques at the amplification stage. Another factor is that the positive control has been damaged due to storage errors. The solution to this problem is to add enzymes and monitor the storage of reagents always at a temperature that is in accordance with the shelf life of the material.

CONCLUSION

Based on the results of the research that have been carried out, several conclusions can be drawn:

1. Detection using the PCR method consists of DNA or RNA extraction, amplification, electrophoresis, and visualization of results using a UV transilluminator.
2. The obstacles found during the detection process are the occurrence of cross-contamination between samples and the surrounding environment and primers that have been damaged due to storage errors.
3. What can be done to prevent WSSV and TSV is to implement biosecurity in ponds, because until now there is still no appropriate treatment.

ACKNOWLEDGEMENT

1. Thank you to BLUPPB Karawang especially to Mrs Atri and Mrs Rohmah as field supervisors.
2. Thanks to the authors and advisors who contributed to the writing of this scientific article

REFERENCES

Alam S. 2017. Deteksi Virus WSSV (*White Spot Syndrome Virus*) Pada Udang vannamei (*Litopenaeus vannamei*). *Skripsi*. Universitas Muhammadiyah Makassar. Hlm. 1- 46.

Chang, P.-S., Chen, L.-J., & Wang, Y.-C. (1998). The effect of ultraviolet irradiation, heat, pH, ozone, salinity and chemical disinfectants on the infectivity of *White Spot Syndrome baculovirus*. In Aquaculture (Vol. 166).

Chen, K. F., Tan, W. S., Ong, L. K., Zainal Abidin, S. A., Othman, I., Tey, B. T., & Lee, R. F. S. (2021). The *Macrobrachium rosenbergii nodavirus*: a detailed review of structure, infectivity, host immunity, diagnosis and prevention. In Reviews in Aquaculture (Vol. 13, Issue 4, pp. 2117–2141). John Wiley and Sons Inc. https://doi.org/10.1111/raq.12562

Denzin, N., & Staak, C. (2000). Fish Immunoglobulin-A Sero-Diagnostician's Perspective. In Bull. Eur. Ass. Fish Pathol (Vol. 20, Issue 2).

Dhar, A. K., Cowley, J. A., Hasson, K. W., & Walker, P. J. (2004). Genomic Organization, Biology, And Diagnosis Of *Taura Syndrome Virus* And Yellowhead Virus Of Penaeid Shrimp. *J. Of vir*. (63) : 353

Fadilah, A. N., & Fasya, A. H. (2021). Examination of *Taura Syndrome Virus* (TSV) in white shrimp (*Litopenaeus vannamei*) and tiger prawn (*Penaeus monodon*) with Polymerase Chain Reaction (PCR) method. IOP Conference Series: Earth and Environmental Science, 679(1). https://doi.org/10.1088/17551315/679/1/012069

Fadlan. F.D. Asep, E. Tantan, A. 2014. Perbandingan Ekstraksi DNA *Salmonella typhi* Dari Kultur Darah Metode Spin Column Dan Alcohol Based. *Jurnal Riset Kesehatan*. Poltekkes Kemenkes Bandung. 11(2)

Hardyat., Slamet, B.P. Alfabetian, H.C.H. (2014). Infeksi *White Spot Syndrome virus* pada udang windu yang dipelihara pada salinitas berbeda. *J.of.Aquac.manage and techno*. 3 (3) : 25 – 34.

IQ2000TM. 2018. WSSV Detection and Prevention System *White Spot Syndrome Virus* (WSSV). World Organization for Animal Health. 1 - 26

Joseph, A., & Philip, R. (2007). Acute salinity stress alters the haemolymph metabolic profile of Penaeus monodon and reduces immunocompetence to *white spot syndrome virus* infection.

Aquaculture, 272(1–4), 87–97. https://doi.org/10.1016/j.aquaculture.2007.08.047

Khofifah, A., Abida, I. W., & Khusna, A. (2023). Pemeriksaan WSSV (*White Syndrome Virus*) Dengan Uji PCR (Polymerase Chain Reaction) Pada Udang Vannamei (*Litopenaeus vannamei*) di UPT Laboratorium Kesehatan Ikan dan Lingkungan, Pasuruan Jawa Timur. Juvenil:*Jurnal Ilmiah Kelautan Dan Perikanan*, 4(2), 142–151. https://doi.org/10.21107/juvenil.v4i2.16462

Kilawati, Y. 2015. Pengaruh Serangan WSSV Terhadap Morfologi, Tingkah Laku Dan Kelulushidupan SPF Udang Vannamei Indonesia Yang Dipelihara Dalam Lingkungan Terkontrol. berkalahayati.org. Brawijaya University. 1 – 5.

Lee, D., Yu, Y. Bin, Choi, J. H., Jo, A. H., Hong, S. M., Kang, J. C., & Kim, J. H. (2022). Viral Shrimp Diseases Listed by the OIE: A Review. In Viruses (Vol. 14, Issue 3). MDPI. https://doi.org/10.3390/v14030585

Monaghan, S. J., Chee, D., Adams, A., Bergmann, S. M., Chong, S. M., Chen, J., & Thompson, K. D. (2022). Serological analysis of historical field samples reveals major inconsistency between PCR and antibody ELISA for establishing KHV infection status of groups and individual koi. Aquaculture, 546. https://doi.org/10.1016/j.aquaculture.2021.737336

OIE. 2018. Infection with *Taura Syndrome Virus*. In Manual of Diagnostic Tests for Aquatic Animals; OFFICE International des Epizooties: Paris, France, pp. 1–18.

Sanjuktha, M., Stalin Raj, V. S., Aravindan, K., Alavandi, S. V., Poornima, M., & Santiago, T. C. (2012). Comparative efficacy of double-stranded RNAs targeting WSSV structural and nonstructural genes in controlling viral multiplication in Penaeus monodon. Archives of Virology, 157(5), 993–998. https://doi.org/10.1007/s00705-012-1258-2

Satheesh Kumar, S., Ananda Bharathi, R., Rajan, J. J. S., Alavandi, S. V., Poornima, M., Balasubramanian, C. P., & Ponniah, A. G. (2013). Viability of *White Spot Syndrome Virus* (WSSV) in sediment during sun-drying (drainable pond) and under non-drainable pond conditions indicated by infectivity to shrimp. Aquaculture, 402–403, 119–126.https://doi.org/10.1016/j.aquaculture.2013.04.001

Sudha, P. M., Mohan, C. V, Shankar, K. M., & Hegde, A. (1998). Relationship between *White Spot Syndrome Virus* infection and clinical manifestation in Indian cultured penaeid shrimp. In Aquaculture (Vol. 167).

Tang, K. F. J., Wang, J., & Lightner, D. V. (2004). Quantitation of *Taura syndrome virus* by real-time RT-PCR with a TaqMan assay. *Journal of Virological Methods*, 115(1), 109–114. https://doi.org/10.1016/j.jviromet.2003.09.021

Wertheim, J. O., Tang, K. F. J., Navarro, S. A., & Lightner, D. V. (2009). A quick fuse and the emergence of *Taura syndrome virus*. Virology, 390(2), 324–329. https://doi.org/10.1016/j.virol.2009.05.010