Transmission of White Spot Syndrome Virus and Possible Use of Physical Barrier as Preventive Measure

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Abstrak

Transmisi White Spot Syndrome Virus
dan Penggunaan Barier Fisik Sebagai Upaya Pencegahan

Penyakit bercak putih viral hingga saat ini masih menjadi masalah dalam budidaya udang. Munculnya penyakit tersebut diikuti kematian massal, sehingga menimbulkan kerugian besar. Penyakit yang disebabkan white spots syndrome virus (WSSV) menular cepat dari satu petakan tambak ke petakan lain. Penelitian bertujuan melakukan uji kuantitas WSSV pada transmisi virus baik melalui air dan kohabitasi. Metode penelitian adalah bioassay dilakukan skala laboratorium. Penularan melalui air disimulasi pada akuarium disekat dengan 3 jenis kasa berukuran pori berbeda, yaitu 300µ, 700µ dan 2 mm. Kohabitasi dilakukan dengan memelihara udang terinfeksi WSSV secara buatan dengan udang dan moluska sehat. Hasil penelitian didapatkan bahwa WSSV menimbulkan infeksi pada udang sehat yang ditempatkan terpisah dari udang sakit menggunakan sekat kasa. Virus bercak putih juga menular secara kohabitasi udang sakit dengan udang sehat baik dari udang windu ke udang windu (sejenis) maupun udang windu ke udang vannamei (berlainan jenis). Hasi penelitian menunjukkan bahwa trisipan bukan karier WSSV, karena tidak menularkan. Analisis LT_{-50} (lethal time 50%) didapatkan bahwa udang yang diuji tantang WSSV melalui inkubasi dengan air mengandung ekstrak WSSV didapatkan konsentrasi 2,75x102 WSSV copy.mL^{-1} menyebabkan kematian 50% dalam waktu 108 jam atau hampir lima hari. Penggunaan kasa putih meskipun tidak sepenuhnya menahan, dapat menghambat sebagian transmisi WSSV. Hasil kajian memberikan gambaran tentang kecepatan penyebaran WSSV di lingkungan budidaya udang serta memberikan panduan bagaimana mengendalikan WSSV.

***Kata kunci**: penyakit, transmisi WSSV, udang, kohabitasi, trisipan*

Abstract

White spot viral disease has devastated shrimp industry in Indonesia. The emergence of this disease is always followed by massive death causing huge losses. Disease is caused by a virus namely White spots syndrome virus (WSSV) is rapidly transmitted from one pond to other ponds. This study aims to quantify WSSV upon transmission process at different route of transmission either through water, the cohabitation ant to cerithidae. A model has developed to fascilitate transmission through water. Aquarium capacity of 60 liter use in this research, each made into two compartment with separation by 3 different screen with pore sizes, 300µ, 700µ and 2 mm. Healthy tiger shrimp Penaeus monodon, reared in one compartment and artifitially diseased shrimp in another compartment. Cohabitation was done by rearing healthy shrimp of tiger shrimp and vannamei shrimp together with artifially infected shrimp with WSSV. For transmission through snails, snail fed with infected shrimp and reared together with infected live shrimp. Transmission also done through bathing healthy shrimp into water contained WSSV extract. The result showed that WSSV is able to cause infection in healthy shrimp eventhough are spaced apart from diseased shrimp using different mesh size screen. White spot virus can also be transmitted by cohabitation from diseased tiger shrimp either to tiger shrimp (same species) or to vannamei shrimp (different species). The result showed that snail is not a career for WSSV. LT-50 of challenge of shrimp with WSSV through incubation with water contained of WSSV extract found that innocula at concentration 2,75 x 105 WSSV copy.μl^{-1}

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causing mortality at 50% within 108 hours. White screen, eventhough not fully efective, but still can retarded WSSV transmission. Result of study provide a greater understanding of how the virus be transmitted in the shrimp farm, and as guidance strategy in controlling in shrimp aquaculture.

Keywords: *disease, transmission of WSSV, cohabitation, shrimp, snail*

Introduction

Shrimp farming industry is still promising until now, because of the potential market (Mohan *et al.,* 2008). The price of shrimp is also relatively higher than other fisheries product beside market demand is also stable (Oktaviani and Erwidodo, 2005). Increased production through shrimp farming is still being encouraged, because the land to increase shrimp production is still quite widely available, amounting to 682 725 ha (Statistik Perikanan, 2010). Government through the fisheries industrialization program has also determined that shrimp production should be improved, including farm demonstration activities (demfarm).The main obstacle to the cultivation of shrimp is white spots disease cause by the White spot syndrome virus (WSSV). Disease-causing agent is a virus of the genus Whispovirus, Nimaviridae family. In some cases of disease outbreaks, mortality can reach 100% of the population. Among virus infecting shrimp, WSSV is a larger virus that has the size of 300 mμ.

The white spot syndrome virus was first discovered in Japan in 1993 (Nakano *et al*., 1994; Chou *et al*., 1995) and then appeared attacking tiger shrimp in Thailand 1994 (Wongteerasupaya *et al*., 1995; Lo *et al*., 1996), and quickly spread to the ASEAN countries, then spread to America because of the importation of frozen shrimp containing virus (Nunan *et al*., 1998). Diseased shrimp characterized by clinical symptoms such as white patches on the carapace.

Transmission of the virus can occur through several types of crustaceans and non-crustaceans crayfish career: *Trachipenaeus curvirostris*, *Metapenaeus ensis*, and *Exopalaemon orientalis*, (Chang *et al*., 1998), also attacked the lobster species *Panulirus penicillatus* and *Panulirus versicolor.* Freshwater shrimp *Macrobrachium* sp. is also can be transmitted by virus without any sign of disease. In addition, WSSV was also found on the marine worm *Nereis* sp. (Vijayan *et al*., 2005).

This research determines Lethal Time 50% of WSSV to tiger shrimp (*Penaeus monodon*), transmission of diseased to healthy shrimp through water, cohabitation and either ingestion or cohabitation by snail and possibilities use of physical barrier to prevent transmission.

Material and Methods

The equipment used to conduct research is aquarium 60 cm x 30 cm x 30 cm in size. Each aquarium was separated into two compartment by a different mesh size of screen which are white (300 μm), green (700 μm) and black (5 mm) (Figure 1). Testing of molecular biology performed by using thermocycler machine (Thermo Scientific), set of micropipette, centrifuge and Uvi-vis spectrophotometer (Schimadzu), Gel doc for PCR documentation. Applied Biosystems 7500 Fast Instrument Real Time also use for Quantitative analysis on the WSSV extract.

Tiger shrimp (*Penaeus monodon* Fab.) used for testing is specific pathogen free status (SPF) for WSSV produced in the Main Center for Brackishwater Aquaculture Development (BBPBAP) Jepara, 5 grams in size, and White leg shrimp (*Litopenaeus vannamei*) at same size. Virus isolates is originated from naturally infected shrimp with WSSV, collected from ponds with white spot viral disease based on diagnosis by PCR, and has been kept in -80^{o}C deep freezer. Other material are includes material for conducting PCR, electrophorezing system and histopathological work.

Transmission through cohabitation shrimp with shrimp

Tiger shrimp, SPF (Specific pathogen free) status, size of 5 grams, obtained from BBPBAP. Virus isolates is a collection from WSSV infected ponds. Previously healthy tiger shrimp artificially infected by feeding on naturally infected shrimp. Subsequently infected shrimp were then place in aquarium together with healthy tiger shrimp, vannamei shrimp and cerithide. Observations were base on death after 1, 2, 4, 6, 12, 24, 48, 72, 96 hours post feeding, death shrimp were then checked for PCR. Survivor shrimp after 96 hours were processed for histopathology examination.

Ingestion of WSSV infected shrimp by cerithide snail

Cerithide, a marine snail taken from shrimp pond, were placed in aquaria size 60 liter. Previously, aquarium filled with sand substrate at thickness of 5 cm, filled with water to a depth of 10 cm. Cerithide then were placed into aquarium as

many as 20 snail/aquarium. Snails were fed with WSSV naturally infected shrimp. Analysing of WSSV infections were performed after 120 hours of post feeding by PCR and histopathology.

Transmission through immersion

Gill of WSSV infected shrimp used in previous treatments is used as source of virus. A total of 0.1 g of gill is place in a microtube containing 900 mL of sterile seawater, and homogenized. After homogenization, solution was then centrifuged at 3000 rpm for 20 min at 4^{o}C, and the supernatant was taken. Supernatant was centrifuged again at 8000 rpm for 30 minutes. Finally supernatant was filtered with a 0.45 µm filter. Filtrate containing virus was the dilutied in series of 1000, 10.000, and 100.000 as treatment I, treatment II and treatment III, respectively. Healthy tiger shrimp as many as 8 shrimp are bath in a solution containing WSSV at different concentration as mentioned previously for 30 minute. Furthermore, shrimp transferred to 60-liter tank containing sterile seawater and equiped with aeration system. The treatment is also accompanied by controls, which are shrimp soaked in sterile seawater. Shrimp mortality was observed at 24 hours, 48 hours, 72 hours, 96 hours and 120 hours post-treatment. A total of 4 shrimps were then taken up to 120 hours when shrip still alive and tested for both PCR and histopathology.

Use of physical barrier

Eight shrimp is maintained in the same aquarium with other eight shrimp artificially infected by feeding carcass of naturally infected shrimp with WSSV. The two groups were separated by screen at different mesh size, which is white colour, medium green color and the size of the biggest black one. Shrimp mortality was observed at 1, 2, 4, 6, 12, 24, 48, 72, 96 hours post-infection. Checking of infected shrimp done by PCR. Shrimp which are still alive up to 96 hours performed fixation with Davidson solution for further histopathological procedures.

Biomolecular and histopathological examination

Polymerase Chain Reaction examination was done by firstly extracting DNA from 0.2 gram tissue of swimming legs, gills and muscle in the micro tube the homogenized with a mortar. Cerithide tissue was done by crushing shell, then the meat was treated like those of shrimp. Digestion buffer (50 mM Tris-Cl pH 7.4, 10 mM NaCl, 10 mM EDTA, 1% SDS) and freshly added 400 µl proteinase K was added to homogenizing tissue to a final concentration of 300 µg.ml^{-1}. Tubes were incubated in a shaking incubator or incubator adjusted at 37^{o}C for 1 hour. Equal volume of phenol was added and then centrifuged at a speed of 11,600 g for 5 min. The aqueous phase is separated and transferred to a new Eppendorf, then added phenol CIAA (Chloroform Isoamyl Alcohol) (25:24:1) with equal volume of phenol and centrifuged divorteks with the speed of 11,600 g for 5 min. Aqaeous phase are carefully taken and transferred to a new tube, then added 1/10 volume of 2 M Na-acetate pH 4.8 and 2x volume of cold absolute ethanol and incubated in the freezer, tube twisted around, and centrifuged at 11,600 g for 5 speed minutes to pellet precipitate, the solution was poured subsequently disposed of by way of air-dried. The final stage is dissolving pellets in TE buffer (100 mM Tris pH 8.0, 0.5 mM EDTA pH 8.0). DNA concentration was measured by optical density (OD, optical density) at a wavelength of 260 nm and 280 nm. DNA concentration is calculated as follows: level= value OD x dilution factor x 50 ug.ml^{-1}. Furthermore, empirically, DNA levels were also observed by electrophoresis on 0.8% agarose gel. Determination of DNA concentration = value OD x dilution factor x 50 ug.ml^{-1}. Furthermore, empirically, DNA levels were also observed by electrophoresis on 0.8% agarose gel.

DNA amplification was done based on Lo *et al*. (1996) protocol (OIE) using a set of forward Primer, 146 F1, 5'-ACT ACT CTA AAC TTC TCT AGC AG -3 ', and riverse: 146 R1, 5'-TAA TGC GTT CTT GGG TGT AAT ACG A-3', while for nested primers used 146 F2: 5 '- ACT GCC CCT GTA ATC TCC TCC A - 3' and 146 R2: 5 '- TAC GGC TGC TGC AGC ACC TTG T - 3'. Amplification protocol for implementation is comprised of a hot start, heating at 94^{o}C for 4 min, annealing temperature at 52.5^{o}C for 1st step, and 54^{o}C for 2nd tep, for 1 min and extension 72^{o}C for 2 minutes. Subsequently a total of 39 cycles with each 94^{o}C for 1 min, 55^{o}C for 1 min and 72^{o}C for 2 minute and final extention at 72^{o}C for 5 minutes. PCR products is 1447 bp for first step, and 941 bp for second step.

Quantitative analyses were performed using Real-time quantitative analysis using qRT-PCR, Applied Biosystems 7500 Fast Instrument. Reagent and protocol for performing qRT-PCR were also provided by Applied Biosystem.

Results and Discussion

Transmission of WSSV through different species of shrimp and snail and mode of route are shown in Table 1. Water quality is monitore during commencing trial. Water quality parameters showed in good condition, such as dissolved oxygen (DO) is greater than 4 mg.L^{-1}, temperature of about 27^{o}C and pH 7.8.

Transmission in cohabitation and ingestion

Results of cohabitation by culture of healthy shrimp together with artifitially infected shrimp in one container without insulation for 24 hours was found that, healthy shrimp became sick and infected with WSSV after rearing with infected shrimp. Snail (Cerithidae) reared together with artifitially infected tiger shrimp and fed with WSSV infected shrimp carcasses, that cause disease to shrimp, up to 120 hours did not indicate the presence of WSSV infection, which was confirmed by checking using PCR and histopathology (Table 2.).

Lethal Time 50 (LT-50) of WSSV to tiger shrimp

Extraction WSSV from gills and diluted 100 time getting optical density (OD) of 0.693 to 0.646 for the 260 and 280 wavelengths. Based on the calculation, the DNA content of 3000 mg.ml^{-1}. Quantitative analysis were performed on extract by Qantitative Realtime PCR analysis (qRT-PCR, Applied Biosystems 7500 Fast Instrument resulting of 2,75 x 10^5 copy virus.µL^{-1}. Immersion using shrimp gill extracts was found that the extract with 1000 times dilution (final concentration 2,75 x 10^5 WSSV copy .µl^{-1}) caused mortality of 16.7%, 33.3% and 66.7% after 72 hours, 96 hours and 120 hours post-immersion respectively. Treatment with the extract solution at dilution 10,000 times (final concentration 2,75 x 10^4 WSSV copy.µl^{-1}) results in mortality at 8.3% after 120 hours post-immersion. PCR results showed that death shrimp were infected with WSSV. Survivor shrimp bath with extract dilution 1000 time and 10,000 time also found infected by WSSV. Dilution 100,000 times did not cause the death of up to 120 hours post-immersion, and also negative WSSV in PCR test. Based on the figure 3, LT-50 (lethal time at 50%) could be calculated. Challenge with WSSV innocula at concentration 2,75 x 10^2 WSSV copy.ml^{-1} causing mortality at 50% is 108 hours or almost five days Figure 1. Durand and Lightner (2002) during their study on P. vannamei found LT-50 at concentration of 1 x 10^2 copy WSSV.ml^{-1} is 136 hours or day 7.

Use of screen as physical barrier

Result of transmission through water showed that previously healthy shrimp reared together with WSSV infected shrimp eventhough separated by screen turned out to be sick and dying. Testing by PCR of dead shrimp confirmed that it was infected WSSV. Transmission in trial I (white screen, mesh size 300µm) relatively mild compared with the green filter (mesh size 700 µm) and by a black screen having larger porosity (mesh size 5 mm). On trial using black color screen, WSSV could be detected in group of shrimp that previously healthy at either PCR step 1 (2 of 3 aquarium) and step 2 (3 aquarium). Treatment with green screen, WSSV can be detected in a previously healthy group at PCR first step in one out of three aquaria and detected at the second step in the all aquarium. At trial using white screen, WSSV could be detected in second step two of the three aquariums. Results of treatmen are presented in Table 2, which is a summary of the images electrophoresis as shown Figure 2.

Table 1. WSSV transmission test results in cohabitation, shrimp with shrimp, black tiger shrimp White shrimp and tiger shrimp with snail.

Treatment	Replicate	PCR	
		Step 1	Step 2
Cohabitation Monodon --> aritifitially infected monodon shrimp	1	Positive	Positive
	2	Negative	Positive
	3	Negative	Positive
Cohabitation Vannamei --> aritifitially infected monodon shrimp	1	Negative	Negative
	2	Negative	Negative
	3	Negative	Positive
Ingestion Cerithide --> Naturally infected shrimp	1	Negative	Negative
	2	Negative	Negative
	3	Negative	Negative
Ingestion Cerithide --> Naturally infected shrimp	1	Negative	Negative
	2	Negative	Negative
	3	Negative	Negative

Results of the study indicate that WSSV can be transmitted through different mechanism, and water is the most effective way in transmitting the disease. Based on the study, when diseased shrimp rear in the same aquaria with healthy shrimp shrimp in a the same container eventhough it's separated by various sizes of screen, healthy shrimp has become infected. Shrimp bathed in a solution containing virus extracted from WSSV infected shrimp, with a ratio of 0,1 gr (100 µg) of infected carcass per liters also become infected and lead to death at mortality rate of 67% within 120 hours and alive shrimp also has infected. Mortality rate at 8.3% until 120 hours post treatment on bathing shrimp in 10 µg infected carcass in one litre sea water, and alive shrimp positively infected with WSSV. In lesser concentration of infected shrimp produce neither mortality nor infection on healthy shrimp. In the shrimp grow out ponds, the factors that trigger the death of shrimp which are temperature and low oxygen levels. On the condition of the shrimp stress virus will copy itself to reach levels that can cause death.

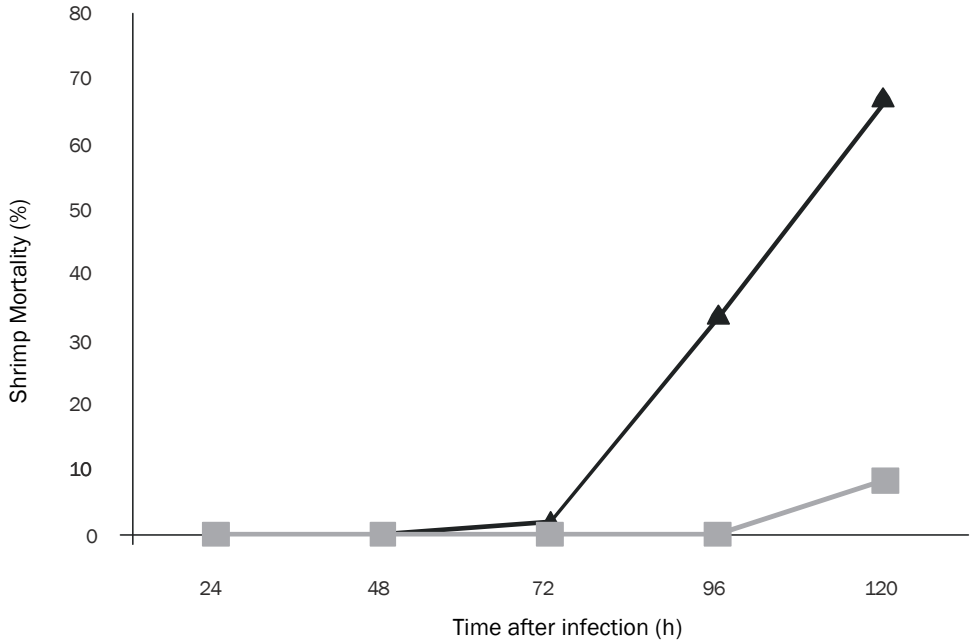

Figure 1. Cumulative mortality tiger shrimp challenge with WSSV innocula by immersion at concentration of 2,75 x 10^5 WSSV copy.μL^{-1} and 2,75 x 10^4 WSSV copy.μL^{-1}. Challenge with lower concentration is not represent in this figure because no mortality observed. Notes: ■ : 2,75 x 10E5, ▲ : 2,75 x 10E4

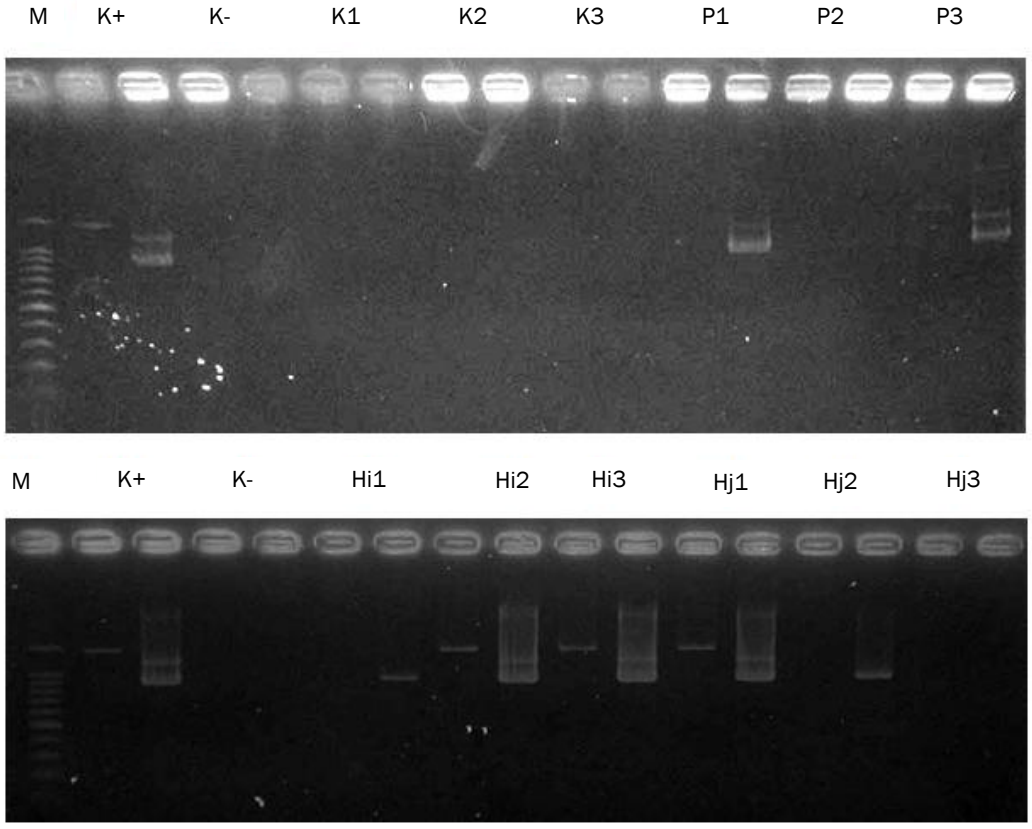

Figure 2. Results of electrophoresis of PCR products on test samples shrimp transmission through water. K + = positive control internal PCR, K-= control negative internal PCR, K= shrimp uninfected, P= treatment with white screen, Hi= treatment with black screen, Hj= treatment with green screen, M= marker 100 bp, number in arabic is replication.

Table 2. WSSV transmission test results conducted on the water through the aquarium container sealing diberik gauze range of pore sizes

Screen	Replicate	PCR Analysis	
		Step 1	Step 2
White (pore: 300 µm)	1	Negative	Positive
	2	Negative	Negative
	3	Negative	Positive
Green (pore: 500 µm)	1	Positive	Positive
	2	Negative	Positive
	3	Negative	Positive
Black (pore: 1 cm)	1	Negative	Positive
	2	Positive	Positive
	3	Positive	Positive
Control	1	Negative	Negative
	2	Negative	Negative
	3	Negative	Negative

The study found WSSV can be transmitted by cohabitation from virus infected monodon to monodon, monodon to vannamei but did not occur in cerithide Cerithide fed with WSSV infected shrimp carcass were not infected, confirmed by PCR test and histopathology. Transmission requires a specific career, in order to become a carrier, the host organism must have a special site for virus attachment. Although WSSV can be transmitted by several species of shrimp, Artemia, a crustasid is not a carrier for WSSV (Waikhom *et al*., 2006) and is also not a viral vector (Hameed *et al*., 2002). Some organisms may be viral vectors, such as birds, worms Polychaeta, but these animals carry the virus only for a limited time. Some species of birds such as herons and gulls can be an intermediary vector for WSSV in stay virulens just a few hours in the digestive tract (van Patten *et al*., 2004).

WSSV transmission is fast during outbreak make the worst situation in the shrimp farm area. In shrimp farming areas, if a pond affected by outbreaks, it is quickly transmitted from one pond to pond surrounding through infected water seepage on the bank. Based on the LT-50, transmission followed by mortality only take 4-5 days at WSSV concentration at 10^2 copy.ml^{-1} water. During outbreak, dead shrimp contained 10^9 to 10^{10} copy DNA.gr^{-1} of shrimp (Oidtmann *et al.,* 2011).

This study also clearly explain that in pond with sand texture allows the intensity of transmission more intense. The more impermeable pond, then the possibility of transmission is also getting lesser. Embankment with holes formed by crabs also make the water flow is greater than one pond to adjacent ponds. Once the shrimp in the pond has been infected by WSSV, when environmental quality conditions decline will induce stress to shrimp, meanwhile virus multiplication will take place followed by shrimp death. Furthermore dead shrimp will be eaten by healthy shrimp resulting in the chain of transmission, but it is also the event of cohabitation, the shrimp were infected with the virus will spread to other shrimp. Although the rate of transmission by cannibalism is much larger than the through cohabitation (Soto and Lotz, 2001), but this cohabitation transmission can reach to a wider area because of infected shrimp alive and still able to reach out to a wider area.

Cerithide that usually found abandon in pond with sandy texture of soils and presumed as carrier like those of worm (Vijayan *et al.,* 2005). Based on this research, it was found that cerithide is not a carrier for WSSV. Proper preparation of pond before stock by drying is an effective way to prevent transmission from one crop to next crop. Cerithide, even it is still alive is not a threat for further crop outbreak. At the time of an outbreak of WSSV in shrimp farming areas, the water in surrounding ponds is rich in virus, so shrimp in ponds is likely be soaked in a basin water containing WSSV at high concentration.

Virus at concentration as low as 10 copy.ml^{-1} may causing mortality and has potential to infect shrimp. At the time of such conditions, transmission intensity will be high so that outbreaks are becoming increasingly rapid.

Table 3. Water quality conditions, including dissolved oxygen (DO), temperature and pH WSSV transmission through water testing performed on the aquarium container sealing diberik gauze range of pore sizes

Screen	Water quality parameter during trial		
	Dissolved oxygen (mg.L^{-1})	Temperature (^{0}C)	pH
White (pore: 300 µm)	4,2 ± 0,2	27,3 ± 0,2	7,8 ± 0,1
Green (pore: 500 µm)	4,5 ± 0,8	27,4 ± 0,4	7,8 ± 0,1
Black (mesh size 1 mm)	5,2 ± 0,7	27,3 ± 0,4	7,8 ± 0,1
Control	4,8 ± 0,5	27,4 ± 0,4	7,8 ± 0,1

Pond located in coastal areas, that is low level is like vessel related each other because ponds separated by sandy textured embankment so that it becomes easier for the transmission. Ponds constructed at a higher tidal (supratidal) and has the texture clayish allow farms relatively safer from the possibility of WSSV transmission, so it's increase the opportunity to success. White screen for screening water even could not totally prevent WSSV, however still prevent partly WSSV to enter system. The mechanism of preventing may trapping floc contain WSSV particles. This finding also relevant to reports by Mohan *et al.* (2008) that considered using screen of 300µm as to protect shrimp pond from WSSV transmission.

Conclusion

WSSV could pass screen of 300 µm mesh size therefor can transmitted through the medium of water. WSSV can be transmitted through cohabitation between shrimp and from shrimp to shrimp vanname. Cerithid is neither infected nor as carrier for WSSV. Proper preparation of pond is an effective way to prevent transmission from one crop to another.

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References

Chang, J-S., H-C Chen & Yu-Chi Wang. 1998. Detection of white spot syndrome associated baculovirus in experimentally infected wild shrimp, crab and lobster by in situ hybridization. *Aquaculture.* 164: 233-242.

Chou, H.Y., C.Y. Huang, C.H. Chiang & C.F. Lo. 1995. Pathogenicity of a baculovirus infection causing white spot syndrome in cultured penaeid shrimp in Taiwan. *Dis. Aquatic. Org*. 23:165-173.

Durand, S.V. & D.V. Lightner. 2002. Quantitative real time PCR for the measurement of white spot syndrome virus in shrimp. *J. Fish Dis.* 25:381-389.

Hameed, A. S. S., B.L.M. Murthi, M. Rasheed, S. Sathish, K. Yoganandhan, V. Murugan & K. Jayaraman. 2002. An investigation of *Artemia* as a possible vector for white spot syndrome virus (WSSV) transmission to *Penaeus indicus. Aquaculture.* 204:1–10.

Lo, C-F, J-H. Leu, C-H. Ho, C-H. Chen, S-E Peng, Y-T. Chen, C-M. Chou, P-Y. Yeh, C-J. Huang, H-Y. Chou, C-H. W & G-H. Kou. 1996. Detection of baculovirus associated with white spot syndrome (WSBV) in penaeid shrimps using polymerase chain reaction. *Dis. Aquat. Org.* 25:133-141.

Mohan, C. V., M.J. Phillips , B.V. Bhat, N.R. Umesh & P.A. Padiyar. 2008. Farm-level plans and husbandry measures for aquatic animal disease emergencies. *Rev. sci. tech. Off. int. Epiz*., 27(1):161-173.

Nakano H., H. Koube, S. Umezaea, K. Momoyama, M. Hirauka, K. Inouye & N. Oseko. 1994. Mass mortalities of cultured kuruma shrimp P. japonicus in Japan in 1993: epizootiological survey and infection trials. *Fish Pathol*. 29:135-139.

Nunan, L. M., B. T. Poulos & D. V. Lightner. 1998. The detection of white spot syndrome virus (WSSV) and Yellow head virus (YHV) in imported commodity shrimp. *Aquaculture.* 160:19-30.

Oidtmann, B. & G.D. Stentiford 2011. White Spot Syndrome Virus (WSSV) concentration in crustacean tissues – a review of data relevant to assess the risk associated with commodity trade. Transbondary and Emerging Diseases. Crown copyright. UK. Pp.: 1–3.

Oktaviani, O. & Erwidodo. 2005. Indonesia's shrimp exports: meeting the challenge of quality standars. Managing the challenge of WTO participation: case study 18. In Gallagher P., P. Low & A. L. Stoler (Eds.): Compilation of 45 case studies which documents among economies in addressing the challeges of participating in the WTO (World Trade Organization), rue de Lausanne 154, CH-1211 Geneva 21, Switzerland.

Soto, M.A. & J.M. Lotz. 2001. Epidemiological parameters of white spot syndrome virus infections in *Litopenaeus vannamei* and *L. setiferus*. *J. Inverteb. Pathol.* 78:9-15.

Statistik Perikanan. 2010. Buku saku statistik perikanan budidaya tahun 2009. Direktorat

Jenderal Perikanan Budidaya. ISSN: 1979-844X.

Van Patten, K. A., L. M. Nunan & D. V. Ligtner. 2004. Seabirds as potential vectors of penaeid shrimp viruses and the development of a surrogate laboratory model utilizing domestic chickens. *Aquaculture*. 241:31-46.

Vijayan, K.K., V. Stalin-Raj, C.P. Balasubramaniam, S.V. Alavandi, V.T. Sekhar & T.C. Santiago. 2005. Polychaeta worms – a vector for white spot syndrome virus (WSSV). *Dis. Aquat. Org.* 63:107-111.

Waikhom, G., K.R. John, M.R. George, M.J. & P. Jeyaseelan. 2006. Differential host passaging alters pathogenicity and induces genomic variation in white spot syndrome virus. *Aquaculture*. 261:54–63.

Wongteerasupaya, C., Vickers, J.E., Sriurairalana, S., Nash, G.L., Akarajamorn, A., Boonsaeng, V., Panyim, S. Tassnakajon, A., Withyachum-narnkul, B. & T.W. Flegel. 1995. A nonoccluded, systemic baculovirus that occurs in cells of ectodermal and mesodermal origin and causes high mortality in the black tiger prawn, *Penaeus monodon*. *Dis. Aquat. Org*. 21(2):69-77.