Replication of white spot syndrome virus (WSSV) in the polychaete Dendronereis spp.

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Short Communication

Replication of white spot syndrome virus (WSSV) in the polychaete *Dendronereis* spp.

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ABSTRACT

This study investigated whether WSSV replicates in naturally infected *Dendronereis* spp., a common polychaete (Nereididae) species in shrimp ponds in Indonesia. To detect WSSV replication, (i) immunohistochemistry (IHC) using a monoclonal antibody against WSSV VP28 protein and (ii) nested RT-PCR using specific primers set for the *vp28* gene to detect WSSV-specific mRNA were applied. WSSV immunoreactive-nuclei were detected in the gut epithelium of the polychaete and WSSV mRNA was detected with nested RT-PCR. This, together with the IHC results, confirmed that WSSV could replicate in *Dendronereis* spp. This is the first report showing that WSSV replicated in a naturally infected non-crustacean host.

1. Introduction

White spot syndrome virus (WSSV), the causative agent of white spot disease (WSD) in penaeid shrimp, belongs to the genus *Whispovirus*, of the Nimaviridae family (Lo et al., 2012). There are two factors that may contribute to the persistence of the virus. Firstly, the virus can persist for a long period in the environment during which susceptible host(s) can be infected. WSSV remained infectious in the seawater up to 40 days (Momoyama et al., 1998). The viral DNA could still be detected in water 20 months after a disease outbreak (Quang et al., 2009) and in pond soil after 10 months of storage at room temperature (Natividad et al., 2008). Secondly, WSSV is unique among shrimp viruses because of its broad host range among crustaceans. The range of reported host species increased from 46 to 94 between 2005 and 2010 (Flegel, 2006; Sanchez-Paz, 2010). In many instances WSSV virulence tended to be lower, sometimes without causing mortality, so the host can survive longer (Esparza-Leal et al., 2009, Chang et al., 2011, Waikhom et al., 2006) and form a WSSV reservoir. These factors may promote horizontal transmission of WSSV in ponds and contribute to its persistence in pond environments.

Polychaetes (Phylum *Annelida*) are common macroinfauna in mangroves (Fujioka et al., 2007) and are an important prey for shrimp (Shishechian et al., 2001). They are often found in and around shrimp pond sites. WSSV was taken in by immersion and accumulated in the gut of the polychaete *Marphysa* spp. (Vijayan et al., 2005). However, whether the virus replicated in the polychaete or was passively carried is a matter of debate. Here, we investigated whether the polychaete *Dendronereis* spp. is a susceptible host of WSSV by showing the presence of WSSV-infected cells in tissue using immunohistochemistry (IHC) and by verifying the presence of WSSV messenger RNA (mRNA) for the major late virion protein, VP28.

2. Materials and Methods

Dendronereis spp. (9–11 cm in length) were randomly collected from a shrimp pond (2.5 ha) in the Semarang district, Central Java, Indonesia. *Penaeus monodon* was cultured traditionally in this pond and the farmer suffered persistent reoccurrence of WSSV infection. The specimens for immunohistochemical (IHC) analysis were collected in January 2010 at which time the farmer was forced to harvest early because of WSSV infection. Seven animals were fixed in Davidson's solution for 48 h and subsequently transferred to 50% ethanol, processed and embedded in paraffin (Ligtner, 1996) for immunohistochemical analysis. Animals used for Reverse Transcriptase-PCR (RT-PCR) analysis ($n = 10$), were collected in February

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2013 at which time the pond contained juvenile *P. monodon* that had been stocked 1 month earlier without signs of WSD.

2.1. Immunohistochemistry (IHC) to detect WSSV infected cells

Nested-PCR was done on the *Dendronereis* spp. paraffin-embedded specimens prior to IHC analysis to verify the presence of WSSV using previously described primer sets for *vp28* (amplicon size 529 bp) (Marks et al., 2003) and a purposely designed *vp28* nested primer set (VP28nest F1: 5'CAT TCC TGT GAC TGC TGA GG 3′; VP28nest R1: CCA CAC ACA AAG GTG CCA AC 3′) (amplicon size 364 bp). The DNA template was prepared using DNeasy Blood and Tissue (QIAGEN) kit following the protocol from the manufacturer. Artificially infected *P. monodon* (positive control) and *Nereis virens* (negative control) were tested alongside WSSV infection tests with *Dendronereis* spp.

The PCR reaction was carried out in a 0.2 ml PCR tube (final reaction volume 25 µl) containing 40–50 ng/µl of DNA, 10 pmol of each forward and reverse primer, 0.4 µl of dNTP (10 mM), 5 µl of 5X PCR buffer (Promega), 1.5 µl of $MgCl_2$ (25 mM) and 2.5 µl of GoTaq Flexy DNA Polymerase (Promega) using Gene Amp PCR System 9600 (Applied Biosystems, Foster City, USA). The 1-step PCR conditions were: initial denaturation (94 °C, 3 min); denaturation (94 °C, 50 s); annealing (50 °C, 50 s) and elongation (72 °C, 1 min) for 30 cycles and a final extension at 72 °C (7 min). One µl of the product of the 1-step PCR was used in the nested-PCR with the same conditions as the 1-step (25 cycles) PCR. The WSSV positive specimens along with uninfected specimens as negative control were further analyzed by IHC.

Paraffin-embedded *Dendronereis* spp. were cut in 5 µm thick sections, mounted on silane-coated slides, deparaffinized, and then rehydrated in a series of ethanol. Endogenous hydrogen peroxidase was blocked by immersion in methanol +0.3% hydrogen peroxide. Tissue was pre-incubated in 5% normal goat serum (30 min), subsequently incubated for 1 h in a 1:100 diluted mouse monoclonal antibody (mAb) solution, specifically reacting with clone C5 expressing VP28 (Anil et al., 2002). The sections were washed twice in phosphate-buffered saline triton (PBS-t), incubated in Goat Anti Mouse-Alkaline Phosphatase (GAM–AP, Dako; 1:200) for 1 h and washed twice in PBS-t. Tissue was incubated in an alkaline phosphatase-buffer (pH 9, 0) (10 min) followed by incubation in the alkaline phosphatase substrate BCIP–NBT (5-bromo-4-chloro-3-indolyl phosphate – nitro blue tetrazolium) until color developed. The reaction was stopped by washing the slides in distilled water.

2.2. RT-PCR to detect WSSV mRNA

Live *Dendronereis* spp was brought to the laboratory, the head was individually stored in Trizol ($n = 10$) at −80 °C for RT-PCR analysis and the rest of the body was checked for WSSV infection by nested PCR. Three WSSV positive individuals were used in the RT-PCR analysis along with one WSSV-negative individual. Total RNA was extracted from 50 mg *Dendronereis* spp. tissue including the head, the first 20 proximal segments and part of the gut using Trizol (Invitrogen). Residual DNA was removed with a DNA-*free* kit (Invitrogen), both following the protocol recommended by the manufacturer, and diluted 50× before proceeding to the next step. First cDNA strand was synthesized using the Superscript III Reverse Transcriptase enzyme (Invitrogen) and an oligo (dT) anchor primer. One µl of the cDNA was used in 1-step RT-PCR reaction and 1 µl of product was used in nested-RT PCR, both using gene specific primer for *vp28* and PCR condition as described in 2.1. The annealing temperature was raised to 55 °C to increase the specificity. WSSV genomic DNA from the infected shrimp was used as positive control and sterile miliQ water was used as no template control for the PCR.18s rRNA of the host (internal control) was detected with primer pair (NVF1 : GTTGATCCTGCCAGTAGTCATATGC; NVR1: TTTCTCATGCTCCCTCTCCGG, amplicon size = 406 bp based on the published sequence of 18s rRNA of *Nereis virens*) (GenBank: Z83754.1). PCR condition for host 18s rRNA was as for *vp28* primer pairs with annealing temperature set at 57 °C for 30 s. The products of RT-PCR were confirmed by sequencing. Sequence data of 18s rRNA of *Dendronereis* spp. has been submitted to the GenBank under accession number BankIt1625623 Dendronereis KC990119.

3. Results

WSSV was detected in paraffin-embedded *Dendronereis* spp. with 1-step PCR in 2 out of 7 individuals and with nested-PCR in 5 out of 7 individuals. WSSV immunoreactive cells were detected only in the stomach and intestinal tissue of *Dendronereis* spp. (Fig. 1), that were positive with 1-step PCR. The nuclei of those cells were enlarged and contained dense and prominent nucleoli and showed strong affinity to the antibody against VP28 as indicated by staining. Infected cells were clearly different from non-infected cells. The latter were homogenous in size and well bordered cells with relatively similar sized and regularly spaced nuclei. These nuclei were not stained with anti-VP28 serum.

Taking advantage of the fact that the *vp28* transcript is polyadenylated (Marks et al., 2003), RT-PCR was used to detect the presence of *vp28* mRNA. Since the expression of VP28 occurs late after infection and is dependent on viral DNA replication, the presence of *vp28* transcripts would signal viral DNA replication. WSSV mRNA encoding the virion envelope protein VP28 was detected in samples of *Dendronereis* spp. (Fig. 2A) as a nested RT-PCR product at the expected size of about 364 bp in 1 out of 3 WSSV-infected *Dendronereis* spp. No PCR product was seen with mRNA template only (without addition of Reverse Transcriptase enzyme) and with no template control (without addition of RNA). The latter served as negative control for the RT reaction and confirmed that the cDNA was amplified only when viral RNA was present. The nested RT-PCR product of *vp28* gene obtained from *Dendronereis* spp. was sequenced and aligned with BLAST (http://blast.ncbi.nlm.nih.gov) against known sequence in the GenBank. It had 99% identity with the WSSV *vp28* gene of an Indonesian isolate (GenBank accession number AY24944) and WSSV genome segment of Thai isolate (AF369029.2, nucleotide 159–491). The host's 18s rRNA was detected in all specimens (Fig 2B) and showed, upon sequencing of the PCR product, 80% identity with 18s RNA of *Nereis virens* and other Nereidae.

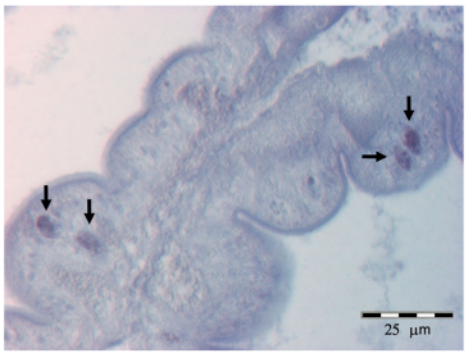

Fig. 1. WSSV-immunoreactive nuclei in the front gut of *Dendronereis* spp. (arrow) and adjacent uninfected cells.

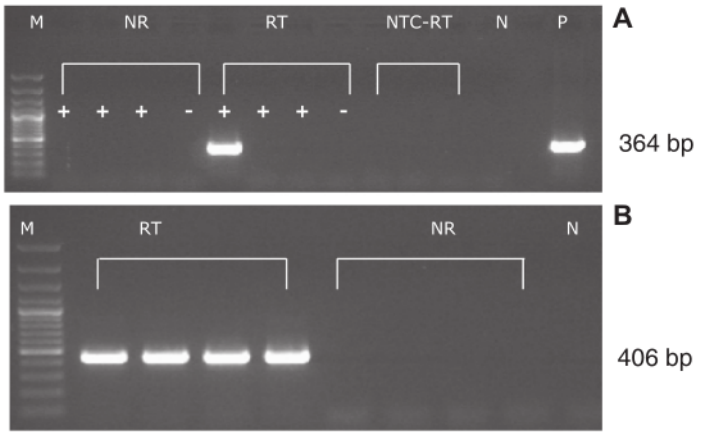

Fig. 2. Nested RT-PCR of the WSSV mRNA of the *vp28* gene (A). One out of three individuals of WSSV-positive-*Dendronereis* spp. showed positive signal. M = Marker (100 bp DNA ladder), NR = No addition of RT-enzymes; RT = with addition of RT enzyme; NTC-RT = No template control for RT step (RNA was replaced with MQ water); + = positive for WSSV with nested PCR; – = negative for WSSV with nested PCR. N = Negative control of PCR; P = Positive control of PCR (DNA of infected shrimp). RT-PCR of messenger of 18s rRNA of *Dendronereis* spp. (B). For explanation see part A.

4. Discussion

Our observations suggest that *Dendronereis* spp. is a propagative host of WSSV. The identification of immunoreactive nuclei in the gut tissue as indicated by IHC and the presence of WSSV-specific mRNA as indicated by the RT-PCR, both support the view that WSSV replicates, at least in some cells, in this polychaete. Despite the difference in sensitivity of the two methods, the results of IHC and PCR converge in their interpretation. WSSV-immunoreactive nuclei were detected only in specimens that were positive with 1-step PCR, indicative of the extent of the WSSV infection in the *Dendronereis* spp. Newly-made WSSV virions accumulate in the nuclei of infected cells (Lo et al., 2012) and VP28 is a major constituent of these virions. Generally, 1-step PCR is positive with heavily infected individuals. So, it can be concluded from the results of the PCR on paraffin-embedded specimens that these individuals contained relatively high concentration of viral DNA, as viral DNA in the paraffin material must have been partially degraded by the chemicals used.

The result of RT-PCR supports the IHC findings. The *vp28* gene transcript was detected in one of three animal tested with nested-RT-PCR indicating the expression level was low. The animals had light infection as shown by nested-PCR. Since mRNA synthesis is an intermediate step in the synthesis of VP28, the presence of mRNA signals late RNA transcription, which can only occur after DNA replication. The fact that complementing results were obtained with naturally infected specimens collected in the same pond three years apart strengthens evidence that WSSV replicated in *Dendronereis* spp.

WSSV morphogenesis occurs in the nucleus of foregut and stomach epithelium of shrimp and crab (Durand et al., 1997). Our results showed that WSSV also infected and replicated in the foregut epithelium of *Dendronereis* spp., despite the fact that *Dendronereis* spp. belongs to a different phylum in the animal kingdom (Annelida). Hence, WSSV has a wider host range than Crustaceans alone and must be considered an even more generalist virus than previously thought.

Dendronereis spp. are widely distributed in the mangrove areas in Southeast Asia (Pillai, 1965; Kumar, 2003; Sarkar et al., 2005) and Africa (Ngqulana et al., 2010) and are natural prey of shrimp in traditional ponds in Indonesia. Even though WSSV replication in penaeid shrimp and decapods has been documented, until today, no replication in planktonic crustaceans and polychaetes has been reported. These organisms were considered to be passive vectors (Escobedo-Bonilla et al., 2008; Stentiford et al., 2009). Our findings showed that *Dendronereis* spp. can also be a propagative carrier. Although the result of Vijayan et al. (2005) suggested that transmission is likely, transmission experiments are still needed to confirm if infected *Dendronereis* spp. also transmit WSSV disease to shrimp when cannibalizing these polychaetes.

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References

Anil, T.M., Shankar, K.M., Mohan, C.V., 2002. Monoclonal antibodies developed for sensitive detection and comparison of white spot syndrome virus isolates in India. Dis. Aquat. Org. 51, 67–75.

Chang, Y., Chen, T., Liu, W., Hwang, J., Lo, C., 2011. Assessment of the roles of copepod *Apocyclops royi* and bivalve mollusk *Meretrix lusoria* in white spot syndrome virus transmission. Marine Biotechnology 13, 909–917.

Durand, S., Lightner, D.V., Redman, R.M., Bonami, J.R., 1997. Ultrastructure and morphogenesis of white spot syndrome baculovirus (WSSV). Dis. Aquat. Org. 29, 205–211.

Escobedo-Bonilla, C.M., Alday-Sanz, V., Wille, M., Sorgeloos, P., Pensaert, M.B., Nauwynck, H.J., 2008. A review on the morphology, molecular characterization, morphogenesis and pathogenesis of white spot syndrome virus. J. Fish Dis. 31, 1–18.

Esparza-Leal, H.M., Escobedo-Bonilla, C.M., Casillas-Hernández, R., Álvarez-Ruíz, P., Portillo-Clark, G., Valerio-García, R.C., Hernández-López, J., Méndez-Lozano, J., Vibanco-Pérez, N., Magallón-Barajas, F.J., 2009. Detection of white spot syndrome virus in filtered shrimp-farm water fractions and experimental evaluation of its infectivity in *Penaeus (Litopenaeus) vannamei*. Aquaculture 292, 16–22.

Flegel, T.W., 2006. Detection of major penaeid shrimp viruses in Asia, a major historical perspective with emphasis on Thailand. Aquaculture 258, 1–33.

Fujioka, Y., Shimoda, T., Srithong, C., 2007. Diversity and community structure of macrobenthic fauna in shrimp aquaculture ponds of the Gulf of Thailand. Japan. Agr. Res. Qarterl. 41, 163–172.

Kumar, R.S., 2003. A checklist of polychaete species some mangroves of Asia. Zoos Print J. 18, 1017–1020.

Ligtner, D.V., 1996. A Handbook of Shrimp Pathology And Diagnostic Procedures for Diseases of Cultured Penaeid Shrimp. The World Aquaculture Society, Baton Rouge, LA, USA, pp. 304.

Lo, C.F., Aoki, T., Bonami, J.R., Flegel, T., Leu, J.H., Lightner, D.V., Stentiford, G., Söderhäll, K., Walker, P.J., Wang, H.C., Xun, X., Yang, F., Vlak, J.M., 2012. Family Nimaviridae. In: King, A.M.Q., Adams, M.J., Carstens, E.B., Lefkowitz, E.J. (Eds.), Virus Taxonomy: Classification and Nomenclature of Viruses, Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier–Academic Press, Amsterdam, pp. 229–234.

Marks, H., Mennen, M., Vlak, J.M., Van Hulten, M.C.W., 2003. Transcriptional analysis of the white spot syndrome virus major virion protein genes. J. Gen. Virol. 84, 1517–1523.

Momoyama, K., Midori, H., Nakano, H., Sameshima, M., 1998. Cryopreservation of penaeid rod-shaped DNA virus (PRDV) and its survival in sea water and different temperatures. Fish Pathol. 33, 95–96.

Natividad, K.D.T., Nomura, N., Matsumura, M., 2008. Detection of white spot syndrome virus DNA in pond soil using a 2-step nested PCR. J. Virol. Meth. 149, 28–34.

Ngqulana, S.G., Owen, R.K., Vivier, L., Cyrus, D.P., 2010. Benthic faunal distribution and abundance in the Mfolozi–Msunduzi estuarine system, KwaZulu-Natal, South Africa. Afr. J. Aquat. Sci. 35, 123–133.

Pillai, T.G., 1965. *Annelida polychaeta* from the Philippines and Indonesia. Ceylon. J. Sci. (Bio. Sci.) 5, 110–177.

Quang, N.D., Hoa, P.T.P., Da, T.T., Anh, P.H., 2009. Persistence of white spot syndrome virus in shrimp ponds and surrounding areas after an outbreak. Environ. Monit. Assess. 156, 107–115.

Sanchez-Paz, A., 2010. White spot syndrome virus: an overview on an emergent concern. Vet. Res. 41, 43.

Sarkar, S.K., Bhattacharya, A., Giri, S., Bhattacharya, B., Sarkar, D., Navak, D.C., Chattopadhaya, A.K., 2005. Spatiotemporal variation in benthic polychaetes (Annelida) and relationships with environmental variables in a tropical estuary. Wetlands Ecol. Managem, 13, 55–67.

Shishechian, F., Yusoff, F.M., Shariff, M., 2001. The effects of commercial bacterial products on macrobenthos community in shrimp culture ponds. Aquacult. Int. 9, 429–436.

Stentiford, G.D., Bonami, J.R., Alday-Sanz, V., 2009. A critical review of susceptibility of crustaceans to Taura syndrome, Yellowhead disease and white spot disease and implications of inclusion of these diseases in European legislation. Aquaculture 291, 1–17.

Vijayan, K.K., Stalin Raj, V., Balasubramanian, C.P., Alavandi, S.V., Thillai Sekhar, V., Santiago, T.C., 2005. Polychaete worms – a vector for white spot syndrome virus (WSSV). Dis. Aquat. Org. 63, 107–111.

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

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PAGE 2

PAGE 3

PAGE 4