Simultaneous Occurrence of The White Spot Syndrome Virus (WSSV) in *Penaeus vannamei, Tegillarca granosa* and *Scylla serrata* from Traditional Shrimp Ponds

Dinda Lintang Sari, Desrina*, Haeruddin

Faculty of Fisheries and Marine Sciences, Universitas Diponegoro
Jl. Prof. Jacub Rais, Tembalang, Tembalang, Semarang, 50275 Indonesia
Email: desrina.1965@gmail.com

Abstract

The White Spot Syndrome Virus (WSSV) is a generalist virus and an important pathogen of white leg shrimp (Penaeus vannamei) farming in Indonesia. The shrimp farming system with inadequate biosecurity like the traditional polyculture system may facilitate the virus to infect the invertebrate live in the pond and expand its host and vector range. White leg shrimp (P. vannamei), blood cockles (Tegillarca granosa), and mud crabs (Scylla serrata) are widely used in polyculture system. Therefore, it is necessary to determine the occurrence of WSSV in these organisms. The aim of this research was to determine whether WSSV existed simultaneously in blood cockles and crabs cultivated with White leg shrimp. The survey was conducted in polyculture ponds in Morosari village and the surrounding area. The characteristic of both ponds was the sandy mud bottom and managed using traditional methods and there were no biosecurity measures implemented. A total of 33 organisms were collected, consisting of 20 blood cockles (T. granosa), 10 white leg shrimp (P. vannamei) and 3 mud crabs (S. serrata) were obtained from two traditional polyculture pond and coastal area of Morosari, Bedono, Demak. WSSV was detected using first step and nested Polymerase Chain Reaction (PCR). WSSV DNA from selected specimens were sequenced and aligned to the published WSSV sequence in the gene bank (NCBI) using BLAST application. The result showed that WSSV existed in the three organisms tested in Morosari area, using first step and nested PCR. There is no clinical sign appearance on each sample. Alignment results showed 100 % identity with published VP28 WSSV protein. All results concluded that WSSV was detected simultaneously in shrimp, blood cockle and crab in the study area.

Keywords: *WSSV, PCR, Penaeus vannamei, Tegillarca granosa and Scylla serrata.*

Introduction

The White Spot Syndrome Virus (WSSV), the causative agent of white spot disease (WSD) was first reported in 1992 in Taiwan, which at that time caused mass deaths in tiger shrimp (*Penaeus monodon*) (Zhu *et al.,* 2019). Then WSSV began to spread to various parts of the world including Asia which found in Korea (Lee *et al.,* 2023), Japan (Hano *et al*., 2024), Thailand (Phiwtong *et al.*, 2025), China (Yang *et al*., 2022), Malaysia (Sahidin *et al*.,2022), India (Vijayan *et al*., 2024) and Indonesia. In Indonesia, WSSV was first detected in Java Island in 1998 with pathognomonic symptoms in the form of white spots on the tiger prawn shrimp carapace and subsequently caused detrimental effects on shrimp culture in Indonesia. Affected farms experience mortality reached 100% in one week after the first clinical signs were observed, resulting in only 20% of farms which was still operating in 1999 (Sunarto *et al.*, 2004). Currently, WSSV still exists in the shrimp pond environment and poses threat to shrimp farming in Indonesia. WSSV is a highly virulent shrimp pathogen that can caused 80-100% mortality within 5 to 10 days after clinical sign (white spots) was noticed for the first time. This pathogen is listed as transboundary disease in WOAH (World Organization of Animal Health, 2022), because it has a negative impact on shrimp farm production, affecting wild aquatic animals, there is no effective control and spread through international trade.

WSSV is the only member of the genus Whispovirus, family Nimaviridae (Wang *et al*., 2019). It is a large virus with a genome consisting of double strain DNA size 120–150 nm diameter capsid and length 270-290 nm. WSSV is a generalist virus infecting a broad range of hosts and vectors that make it difficult to control (Pradeep *et al*., 2012). Unlike specialist pathogens that target specific hosts, WSSV is capable of infecting a broad range of host species (Verbruggen *et al*., 2016). Multi-host pathogens are naturally complex, shaped by the interplay between pathogens, hosts, evolution, environment, and climate. Viewing multi-host pathogens through an ecological perspective provides insights that can lead to a broad range of practical applications. These pathogens have the

*) Corresponding author

https://ejournal.undip.ac.id/index.php/ijms
DOI: 10.14710/ik.ijms.30.1.43-52
Received : 30-12-2024
Accepted : 05-02-2025

potential to affect not only host populations and community dynamics but also the interactions among predators, prey, and competitors (Bowden *et al*., 2013). The reported host, carrier and vector consisted of 50 families of aquatic invertebrates and the majority involved decapod crustaceans, and 13 other species from the non-crustacean species (Desrina *et al*., 2022). Based on (Hoa *et al*., 2012) in extensive ponds in Vietnam with very minimum biosecurity, WSSV exists with high genetic variation which is related to the infectivity of this virus and is transmitted to shrimp and crabs in several cycles.

WSSV infected benthic invertebrates that permanent resident in the shrimp ponds like polychaetes, potentially because of the continuous exposure of WSSV in ponds and the characteristic virus of a generalist nature (Haryadi *et al*., 2014). WSSV infection has caused significant loss in many species of farmed crustaceans, but penaeid shrimps are more susceptible than others (Dey *et al.,* 2020). White leg shrimp (*Penaeus vannamei*) farming is an important aquaculture industry in Indonesia (Arsad *et al.,* 2018), with the production reached 764.240 tons in 2023 (Statistics of the Ministry of Marine Affairs and Fisheries of Indonesia, 2023) and contributed up to 34,57% to Indonesian fisheries exports (Statistics of the Ministry of Marine Affairs and Fisheries of Indonesia, 2022). North coast of Central Java Province has a long history as shrimp farming area in Indonesia. Morosari, Demak is one of many shrimp farming areas in north coast of Central Java Province (Suryono and Rochaddi, 2017) that also severely abraded. The area serves as the natural habitat of blood cockle (*T. granosa*) (Afiati, 2007). Due to recurring disease issues, many farmers chose to farmed shrimp in traditional method using low density in monoculture and polyculture system. Ponds were stocked with white leg shrimp (*P. vanamei*) and blood cockle (*T. granosa*). The traditional pond environment may create conditions that favour spread of WSSV among host(s) and potential host(s) exist in polyculture ponds. Inadequate biosecurity practices (Murachman *et al*., 2010) lead to many WSSV host and vectors like crabs roaming freely in and between ponds shedding the virus.

Study on WSSV infection in bivalve is very limited, may be because bivalve was not regarded as susceptible animal to WSSV infection. Chang *et al*. (2011) reported the mollusc *Meretrix lusoria* was a vector of WSSV. Thus, practicing polyculture blood cockles (*T. granosa*) with white leg shrimp have the potential to transmit WSSV virus between them. Furthermore, wild animal like mud crab (*S. serrata*) also posed risk to be WSSV source in the ponds. According to (Pratapa *et al*., 2023) mud crabs (*S. serrata*) do not show any external signs of infection and may act as carriers and vectors of WSSV transmission to other crustacean hosts, especially to shrimp farms or rearing facilities around that natural habitat. It is necessary to carry out research regarding the presence of the WSSV virus in blood cockles (*T. granosa*), white leg shrimp (*P. vannamei*) and mud crab (*S. serrata*) in polyculture cultivation environments. The objective of this research is to determine the simultaneous occurrence of WSSV in blood cockles (*T. granosa*), white leg shrimp (*P. vannamei*) and mud crab (*S. serrata*) from polyculture pond coastal area of Morosari, Demak, Central Java, Indonesia. To the best of our knowledge, this is the first study to detect WSSV occurrence in these three organisms in this area.

Materials and Methods

Sampling was carried out on April 2024 in Morosari, Sayung, Demak, consisting of three sampling areas: the mud crabs (*S. serrata*) were sampled from the Morosari coastal area while the blood cockles (*T. granosa*) and white leg shrimp (*P. vannamei*) were sampled from two of traditional polyculture ponds (Figure 1).

The first pond (5 ha in size) was used for culturing blood cockles (*T. granosa*) and shrimp (*P. vannamei*) with the density of 4 tonnes. The second pond was used for culturing blood cockles (*T. granosa*) and milk fish (*Chanos chanos*) (3 ha in size) with a density of 2 tonnes. The second pond was known to harbour some wild white leg shrimp (*P.vannamei*) naturally. However, these shrimp were not present at the time of the sampling process. The characteristic of both ponds was the sandy mud bottom and managed using traditional methods including tidal water exchange, no aeration, and no supplementary feeding, relying instead on natural food sources such as phytoplankton and there was no biosecurity measures implemented. Over the past 10 years, the pond was never completely dried, but the sludge was removed annually and placed on the dike. The crabs used in this study were wild animals obtained from the coastal area Morosari, close to both ponds for farmed white leg shrimp (*P. vannamei*)-blood cockles (*T. granosa*) and included an estuary area adjacent to a mangrove area with sandy mud bottom waters.

Shrimp, blood cockles and mud crab sampling

Total of 33 organisms were collected, including 10 blood cockles from pond 1, 10 blood cockles from pond 2, 10 white leg shrimp from pond 1, and 3 mud crabs from the coastal area of Morosari, Demak. The number of specimens collected followed guidelines from previous studies (Bandeira *et al*., 2018, Talukder *et al*., 2021 and Norizan *et al*., 2019). The white leg shrimp measured 7–8 cm in length,

weighed between 2.5 and 3 g, and were 2.5 months old. The stocked blood cockles from pond 1 had an average shell length of 2–3 cm and were 7 months old, while those from pond 2 had an average shell length of 3–5 cm and were 1 year old. The mud crabs weighed approximately 300–400 g. Each organism was placed into separate ziplock bags and labelled with identification codes based on the sampling location and type of sample, stored in a cool box, and transported to the laboratory. Standard handling and storage procedures were followed throughout the sampling process. All organisms were immediately transported to the laboratory and stored frozen at–20°C. Water samples were also collected from each farm to conduct water quality tests, including measurements of pH, salinity, dissolved oxygen, and temperature.

DNA extraction

The total of eight pooled sample groups were examined. (Figure 2). Specimen pools consisted of the following: 10 blood cockles from location 1 (code 1), 10 blood cockles from location 2 (code 2), 10 blood cockle gills from location 1 (code 3), 10 blood cockle mantles and feet from location1 (code 4), 1 mud crabs (gills) from location 3 (code 5), 1 mud crabs (gills) from location 3 (code 6), 1 mud crabs (gills) from location 3 (code 7), and 10 shrimp from location 2 (code 8). According to (Carnales *et al*., 2024) the pooling strategy enhances diagnostic efficiency in low-prevalence disease detection by increasing the number of samples analyzed while reducing reagent consumption and overall testing costs. This method improves surveillance sensitivity and enables large-scale screening with minimal resource.

The whole shrimp, cockles and crabs obtained from field (Figure 2), which have been labelled or coded, are then necropsied. Before starting the necropsy, all necessary tools and materials are prepared and sterilized properly. For virus testing, parts of the following blood cockle organs were used: the gills, mantle, feet, while for the shrimp was following protocol described by Desrina *et al.* (2013) and for mud crab target organ tested was the gills (Pratapa *et al.,* 2023). Furthermore, each of the target organs was removed aseptically from each animal, then rinsed carefully in sterile aged seawater and pooled as explained above. This step ensured that the virus detected by PCR obtained from the cells in the target organs and not from particles that might have been attached on the surface of the organs. The DNA extraction performed immediately using the Wizard® Genomic DNA Purification Kit (Promega), started by crushing the target organ (volume: 20 mg in a 1.5 ml microcentrifuge tube) and the rest of protocol was done following the manufacturer manual instruction. The total genomic DNA concentration was 50-100 mg.µl^{-1}. Then the genomic DNA was stored at -20^{o}C until used.

First step and nested PCR to detect WSSV

This study used 1-step and nested PCR. Nested PCR was used to enhance the specificity and sensitivity of detection, particularly when WSSV DNA was present in low amounts. The two-step nested PCR was performed using two sets of primer pairs (Pereira *et al.,* 2019). First step PCR used primers VP28-F1 (5' - CAC AAC ACT GTG ACC AAG - 3') and VP28-R1 (5'- TTT ACT CGG TCT CAG TGC CAG - 3') with amplicon an VP28-R1 (5' - TTT ACT CGG TCT CAG TGC CAG - 3') with amplicon size 529 bp while nested PCR used primers

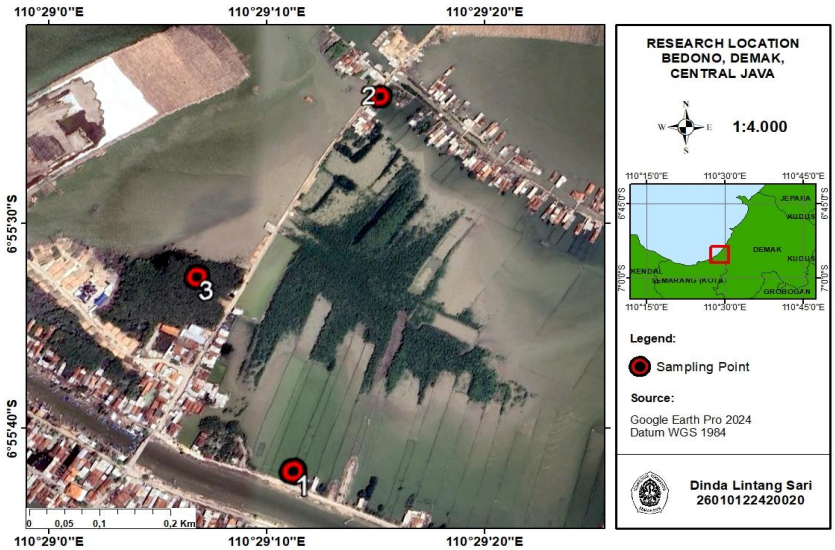

Figure 1. Sampling Location. Location 1 and 2 were abraded shrimp ponds and location 3 is the coastal area which share the same watershed with pond 1 and 2.

WSSV VP28-F1 nested (5' - CAT TCC TGT GAC TGC TGA GG - 3') and VP28-R1 nested (5' - CCA CAC ACA AAG GTG CCA AC - 3') with amplicon size 383 bp (Desrina *et al*., 2013).

The final volume of PCR reaction was 25 ul. The PCR mix consisted of Go Taq Green 12,5 μl, Nuclease Free Water 7,5 μl, 50-100 ng.μl^{-1} of DNA, 10 pmol of each VP-28 forward and VP 28-reverse primer 1 μl. The genomic DNA used for PCR positive control throughout the study was obtained from WSSV naturally infected *P. vannamei* gills. The shrimp showed clinical symptoms of WSSV infection, and experienced a mass mortality outbreak. The nuclease-free water from the Promega kit was used as negative control. The PCR program was the same for both PCR, consisting of denaturation (94°C, 50 sec), Annealing (50°C, 50 sec), elongation (72°C, 1 min) for 30 cycles and a final extension at 72°C (7 min).

Visualization of PCR results

The amplicons obtained from the amplification were analyzed by electrophoresis to show whether or not WSSV was present in the samples that were tested. The PCR products were separated using 1% agarose gel electrophoresis, stained with ethidium bromide (EtBr), visualized under UV Transilluminator, and documented. Selected PCR amplification product was then purified, concentrated, and submitted to a commercial laboratory for DNA sequencing.

Sequencing of the VP28 Gene of WSSV from Blood Cockles (*T. granosa*)., White Leg Shrimp (*P. vannamei*), and Mud Crab (*Scylla serrata*)

Nested PCR products of WSSV DNA from naturally infected WSSV detected ini blood cockles (*T. granosa*)., white leg (*P. vannamei*), and mud crab (*Scylla serrata*) were sent to 1st BASE Laboratory in Selangor, Malaysia, for sequencing. The resulting sequences were aligned to assess homology among the WSSV vp28 sequences obtained in this study and compared with known sequences in GenBank using the BLAST program (www.ncbi.nlm.nih.gov).

Result and Discussion

Clinical sign

Gross sign was an initial step to identify the causative agent of the disease. Based on pond-side sampling and observation there was no clinical sign in blood cockles (*T. granosa*), white leg shrimp (*P. vannamei*) and mud crabs (*S. serrata*) (Figure 2).

The external appearance of white leg shrimp (*P. vannamei*) showed no significant differences compared to healthy shrimp, no presence a white spot or reddish color in the surface of body, hepatopancreas appeared normal with a brown color and showed no signs of significant atrophy. According (Kua *et al.*, 2012) The wild *P. monodon* broodstocks in Malaysia and wild crustaceans in India testing positive for white spot virus, despite showing no visible signs of infection such as white spots on their body surface, has been observed. In subclinical infections, WSSV replication occurs at a lower rate compared to acute infections. The virus primarily replicates in the nucleus of host cells, with early stages. According to (WOAH, 2023) White Spot Syndrome Virus (WSSV) is a highly pathogenic virus affecting shrimp, capable of causing both subclinical (asymptomatic) and clinical infections. In subclinical or light infections, shrimp harbored the virus without exhibiting overt clinical signs, while in heavy infections showed the clear symptoms leading to high mortality rates.

The mud crabs and blood cockles also showed no clinical signs of infection WSSV. Additionally, no small organisms or parasites were observed clinging to the shells of blood cockles (*T. granosa*). Findings of this study suggest that both mud crabs (*S. serrata*) and blood cockles (*T. granosa*) may act as carrier organisms with low levels of WSSV infection, insufficient to cause visible clinical symptoms or mortality. This is supported by PCR detection results,

a b C

Figure 2. The clinical sign, sample of (a) white leg shrimp, (b) blood cockles and (c) mud crab

where the majority of tested samples showed negative in the first step PCR test but positive in the nested PCR test, as shown in (Figure 2). According to Pratapa *et al*. (2023), mud crabs (*S. serrata*) do not exhibit external signs of WSSV infection but may function as carriers and vectors, facilitating the transmission of WSSV to other crustacean hosts. Carrier organisms, while asymptomatic, are capable of transmitting the virus to other organisms Ashari *et al*. (2022). This observation aligns with findings by Maeda *et al*. (2000), reported that wild crabs can serve as carriers and vectors of WSSV, capable of transmitting the virus to various decapod species and crustaceans, including marine and brackish shrimp, freshwater shrimp, crabs, and lobsters. These studies collectively highlight the role of wild crabs and blood cockles as carriers, contributing to the spread of WSSV across aquatic environments.

WSSV detection with first step and nested PCR

The result of the test using 1-step PCR and nested PCR that there are presented in Figure 2. The results showed that only one pool specimen was positive for WSSV with first step PCR, however, the rest of specimen gave positive result on nested PCR confirmed that all pool specimen tested had WSSV.

Based on the electrophoresis results, one positive sample was detected from the gills of blood cockles in pond 2 during the first PCR step, while seven other samples tested positive only in the second

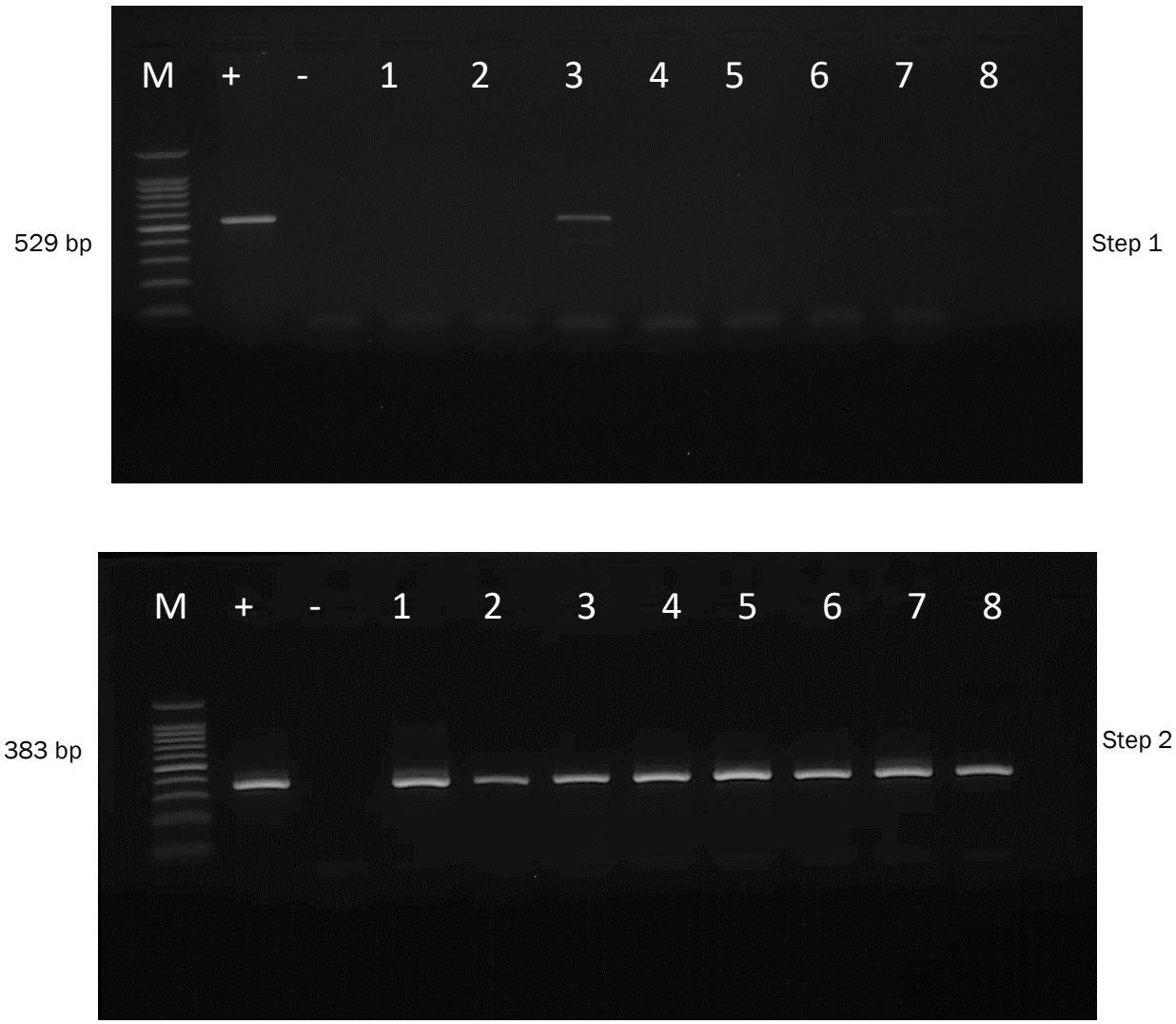

Figure 2. Results of first-step and nested- PCR of Blood cockles (*T. granosa*). mud crab (*S. serrata*) and white leg shrimp (*P. vannamei*) from traditional polyculture pond. Line M= Marker, (+)= Positive control, (-)= negative control, 1= Sample of blood cockles pond A, 2 = Sample of blood cockles pond B, 3 = Sample of blood cockles pond B (gill only), 4= Sample of blood cockles pond B (mantel and feet), 5 = mud crab A, 6= mud crab B, 7 = mud crab C and 8= shrimp

(nested) PCR step. The sample that tested positive in the first step likely represents a heavy infection, whereas those testing positive only in the nested PCR step are indicative of light infections. These light infections are suspected to represent early stages of WSSV infection or carrier status in the sampled organisms. According to (WOAH, 2019) the nested PCR method is a well-established and reliable diagnostic tool under specified conditions. Accurate DNA sampling from recommended organs and precise PCR temperature control, particularly at 55°C for annealing, are essential. Further, nested PCR is highly sensitive and enables the detection of light WSSV infections in wild or cultured shrimp during non-epidemic periods (Onihary *et al*., 2021). Light infections typically occur during the initial phase of WSSV infection when the viral load in host cells is low.

The nested PCR results highlight the potential for asymptomatic carriers to contribute to viral persistence and transmission within a population in pond. Moreover, the light infection is characterized by the virus is actively replicating its DNA and producing new particles, although the expression of its early and late genes remains incomplete. The host's immune system may still exert localized control over the virus, delaying its progression to a more severe stage. According to (Korkut *et al.,* 2018) WSSV infections can manifest as clinical or sub-clinical. During active infections, clinical signs usually appear within 2 to 7 days. In contrast, sub-clinical infections can persist for extended periods without visible symptoms. However, the transition from a sub-clinical to a clinical stage is often rapid and may occur within hours. Additionally based on World Organization for Animal Health (WOAH, 2022), this transition is frequently triggered by stress factors, such as spawning or environmental changes, including fluctuations in rainfall, temperature, and salinity. These stressors can compromise the host's immune defences, allowing the virus to proliferate and cause disease. Indeed, white leg shrimp (*P. vannamei*), blood cockles (*T. granosa*) and mud crab (*S. serrata*) samples that tested positive for WSSV using nested PCR in this study, did not exhibit any visible white spots or reddish coloration. It has been documented that hosts with low levels of infection may not display clinical symptoms, and non-penaeid species like crabs typically experience subclinical infections in their natural habitat. Our finding is inline with result of previous study reported by (Somboona *et al*., 2010), in which shrimp and crab samples that were confirmed positive for WSSV through nested PCR showed no visible signs of white spots. WSSV infection also support the findings of low infection levels or positive nested PCR results, as observed in this study.

The detection of WSSV in blood cockles (*T. granosa*), white leg shrimp (*P. vannamei*), and mud crabs (*S. serrata*) around the same time within multiple ponds at the same location raises significant concerns regarding the increasing number of potential vectors and the subsequent risk of virus spread. This observation highlights WSSV's capacity as a generalist pathogen, capable of infecting a wide variety of hosts or vectors. thereby facilitating its persistence and transmission across diverse aquatic environments. Furthermore, various factors can allow a pathogen to infect multiple host species like genetic changes in animal with natural infection of WSSV. According to Pepin *et al*. (2010) genetic changes in the pathogen may arise due to natural selection or random mutations, enabling it to adapt more effectively to a new host species. It is widely assumed that pathogens with higher mutation rates tend to have greater genetic diversity, increasing the likelihood of them being generalists. High genetic variation may be advantageous for generalist viruses, allowing them to infect a large number of host species, thereby increasing their survival options (Desrina *et al*., 2022). Interestingly, infections have also been increasingly reported in non-crustacean hosts and vectors, such as mollusks (oysters, clams, snails), and polychaetes, annelids (Haryadi *et al.,* 2014), and zooplankton (Porchas-Cornejo *et al*., 2013). Among crustaceans, penaeid shrimp species are highly susceptible to WSSV infection and the resulting disease (Verbruggen *et al.,* 2016).

Furthermore, it was discovered that WSSV can survive for an extended period in drained pond water, even without a host. This condition could further contribute to the spread of the virus. According to (Satheesh-Kumar *et al*., 2015) that WSSV has been found to remain viable in shrimp pond sediments for up to 19 days, even after sun-drying. WSSV can stay viable and infectious in seawater for up to 12 days, albeit at relatively low concentrations. According to (Tendencia *et al*., 2011) that molluscs as filter-feeder organisms can serve as carriers of WSSV by ingesting WSSV particles from the soil and water column, this could allow for the transfer of WSSV to shrimp. Moreover, based on (Min *et al*., 2024) bivalves, including oysters and mussels, can act as vectors for aquatic viruses like as WSSV by absorbing and retaining the virus in their tissues. The accumulation of WSSV in bivalve tissues is significantly influenced by the viral load in the surrounding water. Our findings show that viral load differs between species, with tissue filtration rate, tissue structure, and the digestive gland's ability to retain the virus playing key roles in this process. Further research is required to confirm the role of blood cockles (*T. granosa*) as a vector for WSSV transmission, specifically focusing on the transmission pathway of the virus from blood cockles (*T. granosa*) to white leg shrimp (*P. vannamei*).

Table 1. The results of DNA sequencing analysis using BLAST in the GenBank database for samples of mud crabs, blood cockles, and white leg shrimp.

Code	Specimen	Similarity	Length DNA (bp)	Source	Ref. Number
1_align_crab	*S. serrata*	100%	353	Mexico	FJ756453.1
2_align_blood cockles	*T. granosa*	100%	363	Australian	MF768985.1
3_align_shrimp	*P. vannamei*	100%	359	Bangladesh	MT363795.1

Sequence analysis of WSSV from Mud Crabs (S. serrata), Blood Cockles (T. granosa), and White Leg Shrimp (P. vannamei)

DNA sequence analysis specifically targeting the VP28 gene was conducted by reading the DNA bases using the MEGA 11 software, followed by a BLAST search on the NCBI website, and data matching was performed on GenBank (Table 1).

Sequencing results were obtained from a total of three specimens: two specimens (blood cockle and white leg shrimp) collected from farms, and one specimen (mud crab) collected from a wild coastal area in Morosari, Demak (Table 1). The sequence of VP28 WSSV gene obtained from mud crab (*S. serrata*) in this study, showed 100% homogeneity with the VP28 gene of the WSSV strain isolated from Bangladesh (Accession code MT363795.1; Table 1). Additionally, the result were 100% identical to the corresponding genes of four WSSV strains from different countries worldwide were as follow: Taiwan (Tsai *et al*., 2000), Japan (Kawato *et al.,* 2023), Bangladesh (Talukder *et al*., 2021), and Ecuador (Restrepo *et al*., 2018).

Similarly, sequencing results of blood cockles (*T. granosa*) aligned with the VP28 gene sequences in NCBI GenBank, showed 100% homogeneity with the VP28 gene of WSSV strain Australian (accession number: MF768985.1; Table 1). Sequenced strains were also compared with WSSV strains from other countries, included China (Han *et al*., 2017), Mexico (Ramos-Paredes *et al*., 2011), Brazill (Braunig *et al*., 2011) with similarity was 100%.

Aligment of WSSV DNA from white leg shrimp (*P. vannamei*) exhibited 100% identity with the corresponding gene from the Bangladesh isolate (accession number: MT363795.1; Table 1). Moreover, the GenBank sequences for *P. vannamei* were also 100% identical to data from the United States (Cruz-Flores *et al*., 2020), China (Li *et al*., 2016), Ecuador (Restrepo *et al*., 2018), and Japan (Kawato *et al*., 2023). Based on result sequencing, this confirms that the PCR product is indeed WSSV DNA, however, weather WSSV strains in the mud crabs (*S. serrata*), blood cockles (*T. granosa*), and white leg shrimp (*P. vannamei*) from the same pond are identical needs further study.

Conclusion

Based on findings in present study, it can be concluded that WSSV occurred simultaneously in three organisms studied. that is blood cockles (*T. granosa*), mud crabs (*S. Serrata*) and white leg shrimp (*P. vannamei*) obtained from shrimp ponds and coastal water in the same area without any clinical sign. Alignment of the WSSV VP 28 gene obtained from these three organisms showed 100% identity with published VP28 WSSV protein, confirmed the occurrence of WSSV.

Acknowledgement

The authors thank the Directorate of Research and Community Service, the Ministry of Research and Technology Indonesia for funding this research (Grant number 601-122/UN7.D2/PP/VI/2024).

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