J. Sain Vet. Vol. XXI, No. 1, 2003

THE EVIDENCE OF VIRUS-LIKE PARTICLES IN CRABS (*Scylla* sp.) ISOLATED FROM INSIDE AND OUTSIDE POND SHRIMP INFECTED WITH WHITE SPOT DISEASE

BUKTI DARI PARTIKEL MIRIP VIRUS PADA KEPITING (*Scylla sp.*) YANG DIISOLASI DARI DALAM DAN LUAR KOLAM UDANG YANG TERINFEKSI DENGAN PENYAKIT *WHITE SPOT*

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ABSTRACT

The crabs (*Scylla* sp.) were investigated for the contamination of Whitespot Baculovirus (WSBV) due to they lived inside and outside pond when the disease outbreak. The 6 samples of crabs, all of them were contaminated by WSBV with the founding of virus-like particles in hyperthrophied nuclei and cytoplasm. The size of virus-like particles is 65 $\pm$ 10 nm in diameter and 145 $\pm$ 10 nm in length. We suggest that crabs has a big role in the transmitting the disease in pond an acting as a host for some crustacean virus.

Key words : Crabs, *Scylla* sp., Whitespot Baculovirus

ABSTRAK

Kepiting *Scylla* sp. telah diteliti untuk mengetahui adanya infeksi *Whitespot Baculovirus* (WSBV) karena kepiting tersebut hidup didalam dan diluar kolam saat terjadinya serangan WSBV. Sebanyak 6 sampel dari kepiting, semuanya terinfeksi oleh WSBV dengan diketemukannya partikel mirip virus di hipertropi nukleus dan sitoplasma. Ukuran partikel mirip virus tersebut adalah berdiameter 65 $\pm$ 10 nm dan panjang 145 $\pm$ 10 nm. Diyakini bahwa kepiting mempunyai peran yang besar didalam penularan WSBV di tambak dan berperan sebagai inang dalam virus crustacea.

Kata kunci : Kepiting, *Scylla* sp., *Whitespot Baculovirus*

INTRODUCTION

Recently, many diseases infect fishs and shrimps in shrimp industry. Most of the diseases were coused by viruses. The polluted water made shrimp sensitive to the disease causing outbreak with high mortality. It was though that the disease spread by vertical transmission from broodstock to their offspring through the ovary, fertilized egg and hatched larvae and horizontal transmission between fish where the principal routes of infection are skin abrasions, gill and gut. Recently, the Whitespot Baculovirus (WSBV) was also found in latent infection in freshly caught wild shrimps and crabs (Lou and Kou, 1998). The targeted tissue of WSBV infection originate from both ectoderm and mesoderm. It was very difficult to eliminate crabs live inside and outside pond, some of them may acts a carrier or host of WSBV in ponds.

The economical loss of Indonesian shrimp industries due to some diseases from 1989 to 1992 was 248 million USD or 62 million USD per year (Anonim, 1996). Whitespot Bacilovirus is a virus of double stranded DNA with the size 80 - 270 nm, that infects many species of shrimp cultured in Asia, *Penaeus esculentus, P.kerathusus, P.merguensis, P.monodon, P.penicillatus, P.semisulcaatus* (Lightner and Redman, 1991).

Whitespot Bacilovirus was reported infect shrimp in many countries like China (HHNBV/baculoviral hypodermal and hematopoietic necrosis), Japan, China, Korea (RV-PJ/rod-shape nuclear virus of *P. japonicus*), Thailand (SEMBV/systemic ectodermal and mesodermal baculovirus), Indonesia, Taiwan, Vietnam, Malaysia, India, Texas (WSBV/white spot baculovirus) (Lightner, 1996). The virus was reported in Thailand in 1992 and spread rapidly to Asia and Indo-Pacific regions, because this region is intraregional stocks transportation. The virus was found in European shrimp *P.setiferus* in 1995 that imported from Texas.

The study analysed a virus infection founded in crabs which lived in ponds when the WSBV outbreak occurred. We suggest that crabs were playing a role as a host.

MATERIALS AND METHODS

The crabs *Scylla* sp. used in this experiment was collected from farms located in East Java Province. The crabs lived inside and outside pond when oubreak of WSBV occured. They did not shows white spots in the body of crabs and a confirmation was done by light microscope and rapid field test with Giemsa stain. Sampel from infected gill was taken individually by sterilized canula.

Preparation of specimen for Transmission Electron Microscopic(TEM) studies was carried out according to Bell and Lightner methods (1988). A piece of gill sample from diseased crabs fixed in 2.5 % cold glutaraldehyde in 0.2 M Sorensen phosphate buffer (pH 7.2) for 1 h. After several rinsing in with the buffer solution, the samples were post fixed in 1 % OsO4 for 1 h. Subsequently the tissue were dehydrated with series of ethanol solutions and embedded in Resin. Ultrathin sections were done with ultramicrotome and they were stained in uranyl acetate and lead citrate, and observed with transmission electron microscope.

RESULTS AND DISCUSSIONS

The results of investigation to crabs *Scylla* sp. which lived inside or outside ponds was summarized in Table 1. The infected crabs did not shows any clinicals signs normally as exhibited by shrimp infected WSBV, the sign we can observed was weak movement. But asymptomatic carrier in the laboratory, may positive to the WSBV. This silent carrier is very difficult to observe due to not exhibited any clinical signs. Whitespot disease of *P.penicillatus* and *Macrobrachium rosenbergii* can be observed after removing their carapace, while in crabs such as *Scylla serrata* the gross signs marked by clouded area in the last two segments of the fifth pereiopods (Lo *et al.*, 1996; Peng *et al.*, 1998). Therefore, under stressfull condition, the WSBV is able to cause disease in the crabs. The morphology and the size of virus is presented in Figure 1, two virus were observed in nuclei of infected crabs.

Whitespot Baculovirus is one of the virus to be responsible in collapsed of shrimp industry and hatchery. The shrimp will die between 3-7 days after infection and will cause heavy losses. The virus transmitted may through contaminated crabs or others crustracean organisms living together with shrimp in pond. Maeda *et al.* (1998) have reported that the crabs used in cohabitation experiment died without showing white spot in their bodies. The wild crabs lived inside or outside pond is very difficult to be eliminated. They easily migrate from pond bank to the pond lives together with shrimp. Other host of the virus may also the wild little shrimp accidentally enter inside pond, their number

and movement difficult to be predicted. Some farmer had tried some methods to prevent crabs enter to the pond to minimize the viral contamination. The control of wild crabs by using infectec pond, copepode, pest crabs *H.tridents*, small pest palaemonid shrimp and larvae of ephydridan insect were positive with two step of PCR.

Tabel 1. Detection of crabs by TEM lived inside and outside pond when WSBV outbreak. The crab did not shown any clinical signs of white spot in whole body, but some of them slowing in their movement.

Species	Date of Sampling	Lived inside/ outside pond	Virus particles WSBV-like virus
Scylla sp.	Juli, 2001	inside	+
Scylla sp.	Juli, 2001	ouside	+
Scylla sp.	Agustus, 2001	inside	+
Scylla sp.	Agustus, 2001	ouside	+
Scylla sp.	September, 2001	inside	+
Scylla sp.	September, 2001	ouside	+

the pesticide is only effective in short time, but in the next season the number of crabs will likely not decrease.

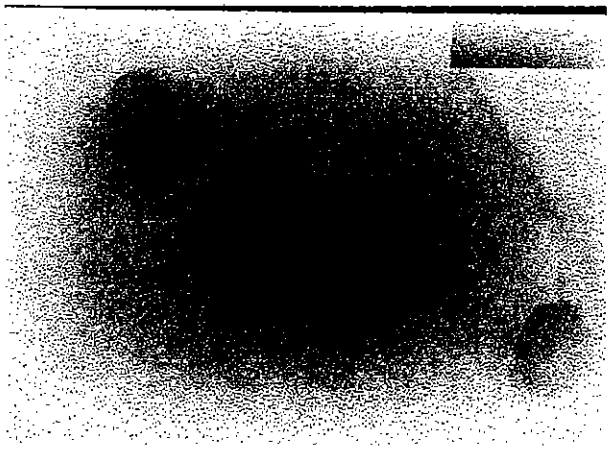

Figure 1. The high magnification of viral particles (arrow) in hyperthrophied nuclei of gill of crabs *Scylla* sp isolated from inside and outside shrimp pond when the Whitespot disease outbreak. Bar = 100 nm

Crabs as hosts of crusctaean virus are well documented by Lo *et al* (1996), they were reported 6 species crabs and shore crab *H.tridents* are shown high incidence of PRDV, indicated that PRDV has a diverse host range. Lo *et al* (1998) reported that WSBV was detected in various crustacean by 1-step or 2-step PCR in Taiwan. They reported that 5 of 11 crabs and 12 of 15 shrimp examined were WSBV positive, cultured arthropods collected from WSBV

We found all 6 samples were WSBV positive in both collected from outside and inside ponds when the WSBV occured. The size of virus particles is 65 $\pm$ 10 nm in width and 145 $\pm$ 10 nm in length. This virus-like particles were seen in hyperthrophied nuclei of crabs gill both inside and outside ponds when the WSBV outbreak. This results though that the virus carried in shore crabs was released into water and infected the healthy shrimp.

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