THE EVIDENCE OF BACILLIFORM VIRUS A CAUSATIVE AGENT OF WHITE SPOT SYNDROME OF WHITE SHRIMP *Penaeus merguiensis*

Hari Suprapto
Department of Fisheries, Faculty of Veterinary Medicine
The University of Airlangga Surabaya
Kampus C Mulyorejo, Surabaya 60115 - Indonesia

ABSTRACT

The rod-shape virus particles were found in the hyperthrophied nucleus and cytoplasm of diseased white shrimp ***Penaeus merguiensis*** *naturally infected by White spot syndrome (WSBV). In natural infection cumulative mortality of shrimp were very high, 95% of population were dead in 3-7 days, and 5% of shrimp population survived. The disease was outbreak in intensive culture farms used the closed circulation sea water system. Others crustacean such as crabs and bentatos were not infected by WSBV at time of sampling. The virus particle were rod-shape ranging from 78 ± 10 nm in diameter and 280 ± 10 nm in length. The pathogenic bacteria mainly dominated by genus* Vibrio *sp were isolated from shrimp.*

Key words: *Bacilliform virus,* ***Penaeus merguiensis****, white spot syndrome*

INTRODUCTION

The virus and bacteria are the main problem in Indonesian shrimp industries causing the collapsed of some hatchery and farms. The virus diseases recently dominated by White spot syndrome (WSBV) appear to be principal disease. WSBV infected shrimp farms since 1996 (Dirjen Perikanan, 1996) continually emerging until today. Affected shrimp 20-30 g in body weight marked by present of white spot in entirely body inside of carapace. In others district in East Java Province the WSBV infected *P. monodon* (Suprapto, 2001) with clinical signs same to white shrimp. The disease shrimp were cultured in intensive farms and used the closed circulation water system. Cumulative mortality will reach 100% within 3-7 days, at least about 5% of population will survived 2-4 weeks after the infection (Chou *et al.*, 1995). WSBV and yellowhead disease (YHV) sometimes occurred together or simultaneously. If the clinical signs appeared, the farmers harvest shrimp immediately to avoid heavy losses. The experiment was deal to finding causative agent of WSBV in shrimp farms in East Java Province.

MATERIALS AND METHODS

The infected shrimp *P. merguiensis* were collected from remote farms in East Java Province which was showed the clinical signs white spot in the entirely body inside carapace. The confirmation of WSBV infection was done by light microscope to find the occlusion bodies. The bacterial, parasitic and fungal inspection was isolated from hepatopancreas, gill and muscle of infected and healthy shrimp and reported separately. Crabs and bentatos were also examined for the infection, subsequently if infected by virus the crabs and bentatos will be submitted for Transmission Electron Microscope (TEM). The sample for histopathological examination were prepared from hepatopancreas, gill, stomach and muscle preserved in cold Davidson's fixative for 24 h and transferred to 50 percent ethanol (Bell and Lighter, 1988). Preparation of specimen for TEM studies were done by the following ways. A hepatopancreas, gill sample from diseased and healthy were shrimp fixed in 2.5% cold glutaraldehyde in 0.2 M Sorensen phosphate buffer (pH 7.2) for 1 h. After several rinses 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 and embedded in Resin. Ultrathin section will cut with ultramicrotome and stained in uranyl acetate and lead citrate and observed with JEOL.

RESULTS

The confirmation WSBV morphology by TEM in the target tissue are showed the presence large rod-shape to somewhat elliptical, non occluded baculovirus. Virus particles found in cytoplasm and nucleus of infected *P. merguiensis* measured to be 78 ± 10 nm in width and 280 ± 10 nm length (Figure 2). In the high magnification the viral particles showed the projectile-like tail and intact envelope. WSBV particles were abundantly present in the hyperthrophied nucleus and cytoplasms of infected shrimp causing the significant damage of cells. The infected shrimp which have same clinical signs were found in others island of Indonesia, may the virus transmitted through contaminated fry from Java. In natural infection shrimp showed the whitespot inside carapas in whole body

(Figure 1), but those signs were not observed in artificial infection of shrimp. In the artificial infection shrimp were abruptly dead without any clinical signs, may the injection doses of virus was very high. The incubation time of viruses is short between 3-5 days, and the mortality will reach 100 percent in days 6-7.

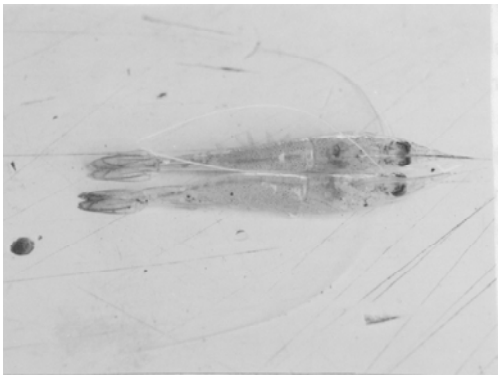

Figure 1. Naturally infected *P.merguiensis* with WSBV, the white spot present inside carapace

Figure 2. Electron micrograph of the gill from WSBV-experimentally infected tiger shrimp *Penaeus merguiensis*. High magnification of virus (arrow) from hyperthrophied nucleus. Bar = 100 nm

DISCUSSIONS

Many new infectious diseases are emerging and causing problems for Indonesian fisheries industry since 1990. The viral disease are the main problem of Indonesian fisheries industry, especially for shrimp culture, and recently the most important are white spot syndrome (WSSV). The outbreak of the diseases were enhance by stress due to high density of shrimp population and polluted water. Naturally infected shrimp showed the white spot inside carapace, but we cannot produce such kind of external signs in laboratory transmission experiments. The natural infection may take a longer time and complex due to abundantly growth factor and mineral in the water, such condition could not produced in the laboratory condition. The light-reddening carapace the only external signs exhibited by the infected shrimp in laboratory transmission experiments. The injected shrimp will dead in 3-4 days after the injection of virus solution, the rapid of onset of WSSV may due to high concentration of virus in the injected solution. The water quality in aquaria is maintenance in normal condition. The clinical signs of naturally infected shrimp are rapid reduction in food consumption, lethargic, lost of appetite, loose cuticle with appearing white spot in diameter 0.5-2.0 mm. Onset of the diseases during 3-4 days with cumulative mortalities almost total population destroyed. At least 5-10 percent of shrimp will survive, and it is interesting phenomenon.

Why the shrimp resist to WSSV infection, have they specific resistance to WSSV. WSSV was found in latent infection in freshly caught wild shrimp and crabs in Taiwan (Lou and Kou, 1998). The production of shrimp was declined in East Java Province due to WSSV infection and the viral agent seems to be highly contagious to shrimp. WSSV infected the intensive culture shrimp that used the closed recirculation system. The suspected carriers are live crabs, bentatos or crustacean inside or outside pond, but those suspected crabs or crustacean when examined by TEM the results are negative against WSSV. Viral particles are not present in the nucleus of gill apparently healthy shrimp. Infected crabs were not exhibited white spot, but moribund crabs will directly dead without left any clinical signs of the disease.

The distribution of White spot disease was reported from China (Baculoviral hypodermal and hematopoietic necrosis/HHNBV), Japan, China, Korea (Rod-shape nuclear virus of *P. japonicus*/RV-PJ), Thailand (Systemic ectodermal and mesodermal baculovirus SEMBV), Indonesia, Taiwan, Vietnam, Malaysia, India, Texas (White spot baculovirus/WSBV) (Lightner, 1996). SEMBV was first reported from Thailand in 1992 (Flegel, 1997) and spread rapidly to region of Asia and Indo-Pacific including Indonesia.

The same morphology of viruses were reported by Suprapto (2001) infected *P. monodon* from East Java Province, they infected shrimp culture intensive which used closed recirculation sea water system. Two year ago between July-December the diseases occurred sporadically in East Java farms. The etiological agent of white spot disease in both of *P. monodon* and *P. merguiensis* appear to be similar viruses, due to morphology and size of the virus are resemblance and they found in the hypertrophied nuclei and cytoplasm of both species shrimp. This finding suggest that WSBV to be caused by same viral agent. Further research need to characterized the virus particle such as the pathogenicity, mode of transmission etc. Although name of viruses were different between countries, but the Indonesian farmers name SEMBV as the etiological agent.

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