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SHORT COMMUNICATION

Pathology Associated with White Spot Virus (WSV) Infection in Wild Broodstock of Tiger Prawns (*Penaeus monodon*)

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Abstrak: Sebanyak enam induk liar udang harimau, *Penaeus monodon*, didapati positif Virus Bintik Putih (WSV) dengan kit pengesanan IQ2000. Histopatologi pada induk udang tersebut menunjukkan jangkitan WSV dengan kehadiran sel hematosit dalam nukleus pada sel-sel epitelium kelenjar antennal, perut dan insang. Induk liar positif dengan WSV ini tidak menunjukkan tanda-tanda penyakit seperti bintik-bintik putih seperti kebiasaan jangkitan WSV. Histopatologi menunjukkan kehadiran hematosit yang terkumpul di sekitar tubul hepatopankreatik akibat jangkitan bakteria. Pengurungan bakteria, pembentukan nodul dan nekrosis juga dikesan pada tubul hepatopankreatik yang turut dijangkiti oleh larva cestoda. Larva Tylocephalum dikesan dalam bentuk kurungan pada organ hepatopankreas dengan dikelilingi oleh sel hematosit. Penemuan ini menunjukkan jangkitan bakteria dan parasit, sebagai tambahan kepada jangkitan virus itu sendiri, boleh menyumbang kepada 80% kadar kematian pada liar induk yang positif dengan WSV.

Kata kunci: Udang Harimau, WSV, Bakteria, Parasit Metazoa, Larva Cestoda

Abstract: A total of six wild broodstocks of tiger prawns, *Penaeus monodon*, were found positive for White Spot Virus (WSV) with an IQ2000 detection kit. Using histopathology, the intranuclear inclusion of haemocyte due to WSV infection was observed in the epithelium cells of the antennal gland, stomach and gills. This result confirmed that the wild broodstocks were positive with WSV without showing any white spot. Additionally, histopathological examination also revealed an accumulation of haemocytes around the hepatopancreatic tubules resulting from bacterial infection. Encapsulation and nodule formation, as well as related necrosis, were also observed around the hepatopancreatic tubules infected with a metazoan parasite. Encysted tylocephalum larval cestodes were observed in the hepatopancreas, with haemocytic aggregation being observed around the infected tubules. These findings showed some bacterial and parasitic infections which, in addition to the viral infection itself, could contribute to the 80% mortality rate in wild broodstocks infected with WSV.

Keywords: Tiger Prawn, WSV, Bacteria, Metazoan Parasite, Larval Cestodes

Currently, wild-caught broodstock are used in hatchery production to supply millions of larvae to stock the ponds. This practice could introduce viral infections into the hatchery population, causing considerable economic losses and limiting the growth of the industry. In Thailand and Indonesia, viral and bacterial infections were found to be important causes of significant pond production losses. Viral diseases, such as White Spot Virus (WSV), and luminous bacterial

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and protozoan infections have frequently been implicated worldwide in disease outbreaks of cultured penaeid shrimp during all phases of production (Brock & Lightner 1990).

WSV is highly virulent, and penaeid shrimp can be carriers of the disease, with transmission of the virus occurring through the ingestion of infected tissue by other animals, disposal of diseased shrimp, and release of pond effluent containing the virus into the sea. Mortality due to WSV in wild broodstock had not been reported in Malaysia since the outbreaks of the virus in 1994 (Hassan *et al.* 1996). In Thailand, the overall prevalence of WSV in wild tiger shrimp (*Penaeus monodon*) broodstock collected from the Gulf of Thailand and Andaman Sea was 3.7% (12/324). In addition to WSV, *P. monodon* could also have shell diseases caused by infection with luminous bacteria or *Vibrio* sp. (causing vibriosis) and contributing to the mortality of cultured shrimp worldwide (Chen *et al.* 2000). Anderson 1988 reported mortality due to rickettsia and bacterial septicemia in juvenile *P. monodon* cultured in Malaysian brackish-water ponds.

In our present study, mortality was observed 3 weeks after transfer of *P. monodon* into the hatchery, with 80% losses. All of the samples tested positive for WSV using an IQ2000 detection kit (GeneReach Biotechnology Corporation, Taiwan). During gross examination, no white spot was observed on the body of any of the broodstock. Water parameters were in the normal range for shrimp culture. The present study aims to investigate the pathology associated with WSV infection in wild broodstock of tiger prawn.

The broodstock specimens were from Johor and were claimed to be Specific Pathogen Free (SPF) stock. Feeding and water management were not changed between when the batch of prawns was brought into the hatchery and when mortality was first noted after 3 weeks. Daily mortality was 6–7 prawns. Water parameters were in the normal range for shrimp culture at the time of investigation.

The specimens obtained were shown to be positive with WSV using an IQ2000^{TM} WSV Detection kit. Detection by the IQ2000 kit showed heavy infection by WSV, and the handling stress during the transportation was unknown. To determine what other pathology was associated with WSV, live positive specimens were processed for histology according to Bell and Lightner (1988). The WSV-infected wild broodstock specimens were injected with Davidson's fixative before fixing in Davidson's solution for 24–48 hours. The standard organs sampled for histology were the head and the third and last segments of abdomen, which were cut and processed by automatic tissue processing system (Leica ASP 300, Leica Microsystems, Germany). The samples were individually embedded in paraffin wax, sectioned into five-micrometer slices, stained with Haematoxylin and Eosin (H&E) and finally mounted with DPX. To test for Gram negative bacterial infection a Gram-Twort stain was applied. The processed slides were examined under a compound microscope (Leica DM5000B, Leica Microsystems, Germany) that was connected to a digital camera (Leica DFC 320, Leica Microsystems, Germany) associated with the computer software Leica QWin (Leica Microsystems, Germany).

Gross observation revealed black patches on the carapaces, erosion on the uropods/appendages and erosion on the antennae of the broodstock (Fig. 1). No white spot was observed on the carapaces. However, the broodstock showed red discolouration on their bodies. Histopathological examination revealed that the WSV-infected tiger prawn broodstock were also infected with bacterial, metazoan and larval cestodes. WSV was evidenced by the intranuclear inclusion of haematocyte observed in the epithelium cells of the antennal glands, stomachs and gills of the two wild broodstock [Figs. 2(a) and (b)]. Cells infected with WSV showed haloed eosinophilic intranuclear inclusions, which developed to become basophilic and filled the moderately hypertrophied nucleus. Changes observed in the WSV-infected prawns when compared to the normal prawns, such as the accumulation of haemocytes around the hepatopancreatic tubules due to bacterial infection, indicated a systemic bacterial infection [Fig. 2(c)]. Further examination using a Gram-Twort stain showed the pink staining that is indicative of gram-negative bacteria [Fig. 2(d)]. Encapsulation, nodule formation and related necrosis were also observed in the hepatopancreatic tubules and were caused by infection with a metazoan parasite [Fig. 2(e)]. Larvae of tylocephalum cestodes were also observed with the haemocytic aggregation surrounding the infected hepatopancreatic tubules [Fig. 2(f)].

Pornlerd *et al.* (1994) reported that 100% mortality could occur less than 7 days after the typical white spot appeared as the first sign of ill health. In our study no white spot appeared, yet 80% mortality was reported and all samples tested using the IQ2000 kit were positive for WSV infection. We believed that infection by bacteria and worms might have weakened the tiger prawn, resulting in subsequent mortality. Bacterial diseases were considered to be a secondary factor related to stress. In addition, the metazoan and larval cestode infections further weakened and stressed the prawns when they were in suboptimal or unstable environmental conditions or high stocking density or were under inadequate management. Clinical vibriosis was found to be the main cause of serious production losses in *P. monodon* grow-out farms in the central shrimp region of Thailand surveyed from Nov. 1988–Oct. 1990 (Nash *et al.* 1992). Bacterial septicaemia associated with *Vibrio* sp. (i.e., vibriosis) has previously been reported as a cause of serious production losses in giant tiger prawn, *Penaeus monodon* Fabricius, reared in semi-intensive to intensive brackish-water ponds in South East Asia (Anderson *et al.* 1988; Nash 1990). In our case, we believed that bacterial and worm infection could be the predisposing factor that stressed and weakened the tiger prawn, leading to the multiplication of WSV in the body that subsequently resulted in the high mortality.

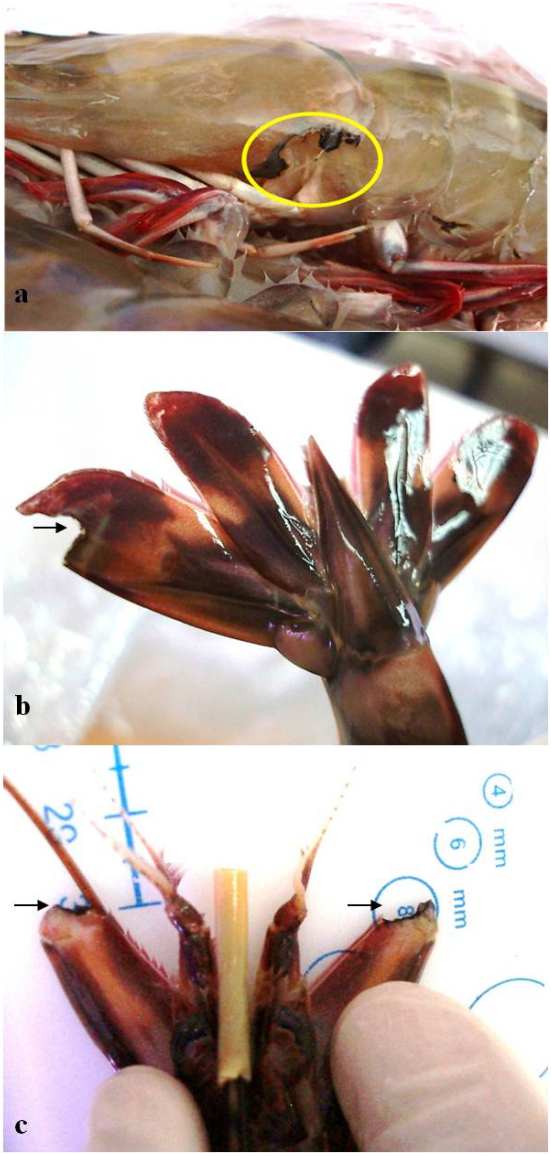

Figure 1: Gross observation of the tiger prawn; (a) black patches on a carapace, (b) erosion on a uropod or appendage (arrow) and (c) erosion on an antenna (arrow).

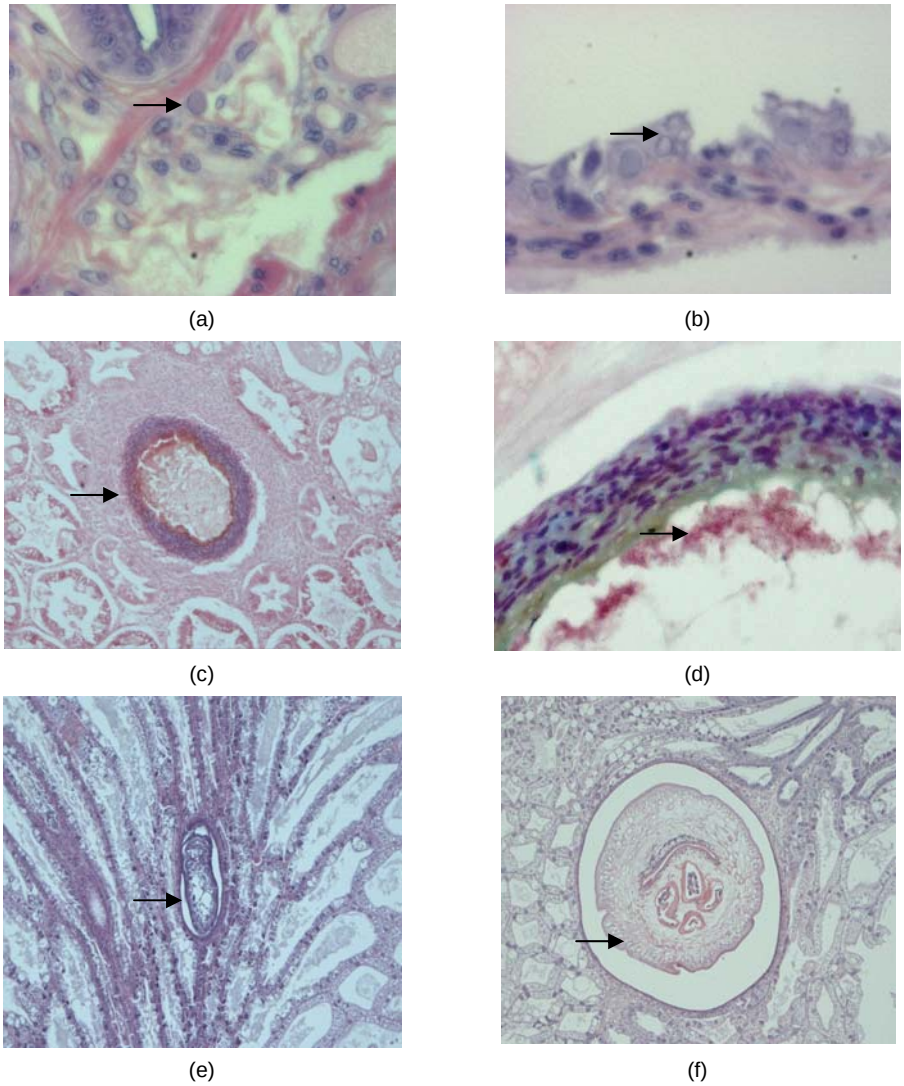

Figure 2: Histological section through hepatopancreatic tissue of an infected WSV-prawn: (a) and (b) an intranuclear inclusion of WSV in the connective tissue of a hepatopancreatic tubule epithelium (arrow) and epithelium of stomach. Magnification: x400, H&E; (c) hepatopancreas showing the massive haemocytic aggregation surrounding the infected tubules and necrosis caused by bacterial infection. Magnification: x200, H&E; (d) the gram-negative bacteria were stained pink using Gram-Twort stain. Magnification: x400; (e) Metazoan parasite infection at the hepatopancreatic tubules (arrow). Magnification: x200. H&E; f) infection by larval cestodes of tylocephalum (arrow) at the hepatopancreatic tubules. Magnification: x200. H&E.

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