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Screening of parasitic and IHHNV infections in wild giant freshwater prawn *Macrobrachium rosenbergii* from Rejang River at Kuching, Sarawak

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Abstract. A preliminary survey of parasitic and infectious hypodermal and haematopoietic necrosis virus (IHHNV) infections in giant freshwater prawn from the Damak Sea of Rejang River, Kuching, Sarawak was conducted. Symptoms of black spots/patches on the rostrum, carapace, pleopods or telson were observed in most of the 107 samples collected. Parasitic examination revealed sessiline peritrichs such as (*Zoothamnium* sp.), nematode larvae, gregarine stage and cocoon of leech with prevalences of 1.2%, 1.2%, 5% and 17% respectively. Under histopathological examination, changes like accumulation of hemocytes around hepatopancreatic tubules due to vibriosis, basophilic intranuclear inclusions in the epithelium and E-cell of hepatopancreatic tubules as a result of HPV were seen through the section. No positive infection of IHHNV was detected in 78 samples. As such, the wild giant freshwater prawns in Damak Sea of Rejang River in Kuching are IHHNV-free though infections of parvo-like virus and bacteria were seen in histopathology.

INTRODUCTION

There has been considerable interest in the farming of giant freshwater prawn *Macrobrachium rosenbergii* in Malaysia due to its relatively fast growth rate and high market price. However, diseases are causing considerable economic losses and limiting the growth of the industry. In Asia, the viral, bacterial and parasitic infections were found to be important causes of significant pond production losses. Among these, viral infection such as infectious hypodermal and haematopoietic necrosis virus (IHHNV) and white tail disease (WTD) are associated with high mortalities at postlarvae and juvenile stages in *M. rosenbergii* (Arcier *et al.*, 1999; Qian *et al.*, 2003; Hsieh *et al.*, 2006). Besides IHHNV, the reported viruses infecting *M. rosenbergii* are *Macrobrachium* hepatopancreatic parvo-like virus (MHPV), white spot syndrome virus (WSSV), *Macrobrachium rosenbergii* nodavirus (*Mr*NV) and extra small virus-like particle (XSV) (Anderson *et al.*, 1990; Peng *et al.*, 1998; Tung *et al.*, 1999). The later two viruses, *Mr*NV and XSV, are causative agents of white tail disease (WTD) which can cause immense economic losses in hatcheries and farms, with 100% mortality (Sahul Hameed *et al.*, 2004).

However there is little or no documentation on the status of diseases of the wild giant freshwater prawn in Malaysia. This information is important as it is the normal practice of Malaysian farmers to use wild prawn as their

broodstock. Based on the previous findings with wild prawns from Perak and N. Sembilan, there is a need to screen the wild giant freshwater prawns in Kuching, Sarawak for parasitic and IHHNV infections as they are also used for breeding purposes.

MATERIALS AND METHODS

Source of Prawn

A total of 107 pieces of live giant freshwater prawn with average size of 45.0 ± 48.0 g weight and 15.0 ± 3.7 cm length were bought from the locals during the period of 1 - 13 May 2009. All the specimens were captured by local fishermen in Damak Sea which is the river mouth of Rejang River in Kuching, Sarawak.

Parasitic examination

Both ectoparasites and endoparasites were examined. Gills, pleopods, stomach and gut were removed and examined under light microscope to confirm the presence of parasitic infections. All parasites noted were recorded and preserved in 70% alcohol for identification. Methods used in preparing specimens for taxonomic studies and identification of parasites followed that of Kabata (1985) while prevalence and mean intensity were based on Margolis *et al.* (1982).

Polymerase Chain Reaction (PCR) of IHHNV infection

Pleopods were removed from giant freshwater prawn and fixed in absolute ethanol before undergoing DNA extraction using lysis buffer from IQ2000™. DNA extraction was performed using IQ2000 kit protocol. The pleopods were dried and homogenized using the micropestle. The DNA pellet was dissolved in 50 µl TE buffer and stored at 4°C until used. The quality of the extracted DNA was checked by electrophoresis gel while the quantity of DNA was obtained by NanoDrop spectrophotometer. DNA from all isolates was then amplified by polymerase chain reaction (PCR). The detection of IHHNV was based on 389 bp using OIE primers. Double stranded amplifications were performed using forward primer (5'- CGG AAC ACA ACC CGA CTT TA-3') and reverse primer (5'- GGC CAA GAC CAA AAT ACG AA-3'). The DNA template, primers and premix taq (Maxime PCR Premix, *i*-taq) were combined and denatured at 94°C for 2 minutes in a thermal cycler with the following condition: denaturing 94°C for 20 seconds, annealing 55°C for 10 seconds, extension 72°C for 30 seconds. The samples were run for 35 cycles and followed with final extension at 72°C for 5 minutes then held at 4°C until they were analyzed. At the completion of PCR, 4 µl of the amplified products were electrophoresed through a 1.5% agarose gel before staining with ethidium bromide and viewed under UV light.

Any positive band from PCR product were purified and sent for sequencing. The partial non-structural protein 1 gene sequences (389bp) of IHHNV were compared with related sequences obtained from the GenBank in NCBI. Multiple alignments were performed with the Clustal X and phylogenetic tree was constructed using MEGA software.

Histopathology

Preparation of specimens for normal histology was done according to Bell & Lightner (1988). A total of 28 wild freshwater prawns were injected with Davidson's fixative before fixing in Davidson's solution for 24 – 48 hours. The specimens were processed by automatic tissue processing (Leica ASP 300) and embedded in paraffin wax which were then sectioned at 5 µm, stained with Haematoxylin and Eosin (H&E) and finally mounted with DPX before being examined it under compound microscope (Leica DM5000B) that was connected to a digital camera (Leica DFC 320) associated with computer software (Leica QWin).

RESULTS & DISCUSSION

Clinical signs of black spots/patches on the rostrum, carapace, pleopods or telson were observed in most of the prawn samples (Figure 1) with prevalence of 67% in Damak Sea of Rejang River in Kuching, Sarawak. Symptoms of black spots/patches on body surface or other parts of body were normally due to melanisation and highly indicative of a disease problem (Bondad-Reantaso *et al*., 2001).

Parasitic examination revealed sessiline peritrichs such as *Zoothamnium* sp., nematode larvae, gregarine stage and cocoon of leech with a prevalence of 1.2%, 1.2%, 5% and 17% respectively (Figure 2 and Table 1). Fouling caused by ectoparasites such as protozoan infection is often observed in larvae of *M. rosenbergii* but are less or not a problem in juvenile or adult stages. A gregarine stage found in the gut with mean intensity of 2.25 could be associated with the main diet of giant freshwater prawn which is the mollusc. Infection of leech cocoon was seen in most of the Sarawak samples with a mean intensity of 107.54 cocoons per prawn. Leeches which act as one of the intermediate hosts in the life cycle of the parasite usually have no significant effect on the adult prawn (Aldrich, 1965; Overstreet, 1972). In the present study, the role played by leech cocoon and the effect of the gregarine stage on adult freshwater giant prawn remains unknown.

Under histopathological examination, abnormal changes like accumulation of hemocytes around hepatopancreatic tubules due to vibriosis, basophilic intranuclear inclusions in the epithelium and E-cell of hepatopancreatic tubules as a result of HPV were observed (Figure 3). Encapsulation and nodule formation and related necrosis were also seen in the hepatopancreas of the affected giant freshwater prawn with bacterial infections. Extensive hepatopancreatic tubular necrosis also occurred.

No positive IHHNV infection was detected from the 78 samples. This indicated that the wild giant freshwater prawns from Damak Sea of Rejang River in Kuching are IHHNV-free though inflicted with HPV and bacterial infections.

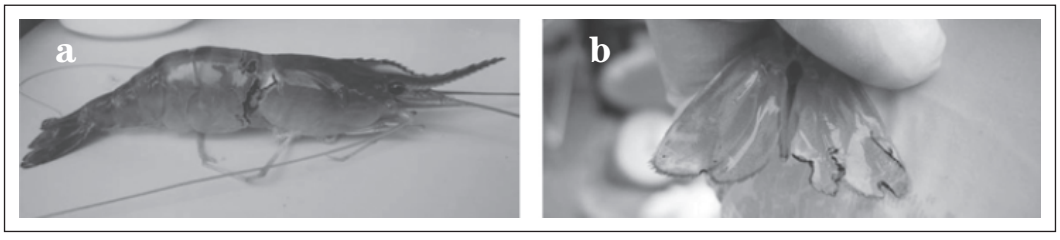

Figure 1. Symptom of black spots/patches on the examined sample
(a) Body (b) telson.

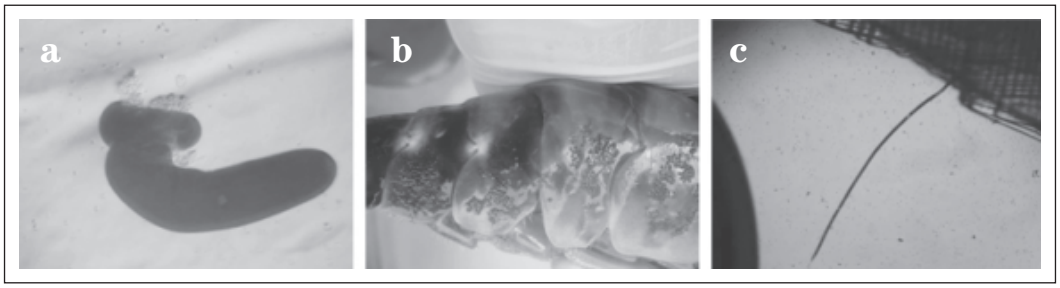

Figure 2. Parasite found in giant freshwater prawn at Damak Sea of Rejang River
(a) Gregarine stage, (b) leech cocoons and (c) nematode larvae.

Table 1. Prevalence and mean intensity of parasites found in giant freshwater prawn at Damak Sea of Rejang River

Parasite	Organ	No. infected / No. examined, (Prevalence)	Mean intensity
Zoothamnium sp.	Gills	1/78, (1.2%)	1.00
Nematode larva	Gills	1/78, (1.2%)	1.00
Gregarine stage	Gut	4/78, (5%)	2.25
Cocoon of leech	Carapace	13/78, (17%)	107.54

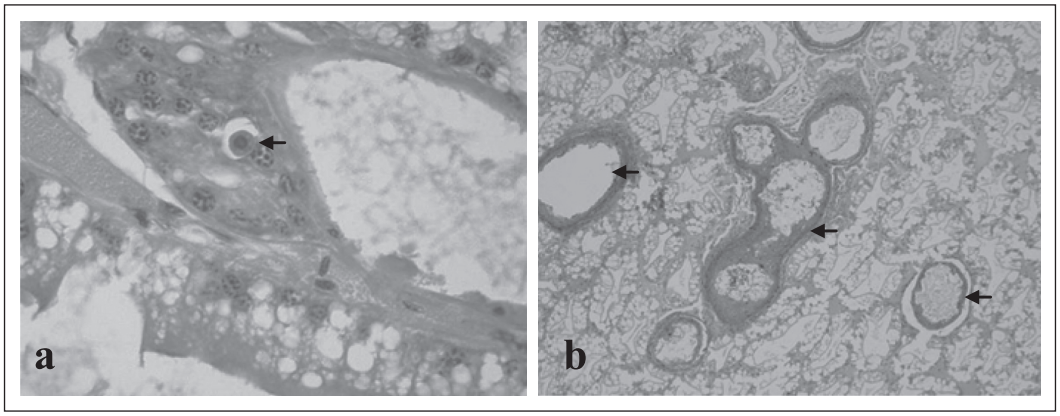

Figure 3. Histological section through hepatopancreas tissue. H & E (a). Infected histological section of septic hepatopancreas showing the HPV (arrow) x200. (b). Massive haemocytic aggregation surrounding the infected tubules and necrosis due to bacterial infection (arrow) x200

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